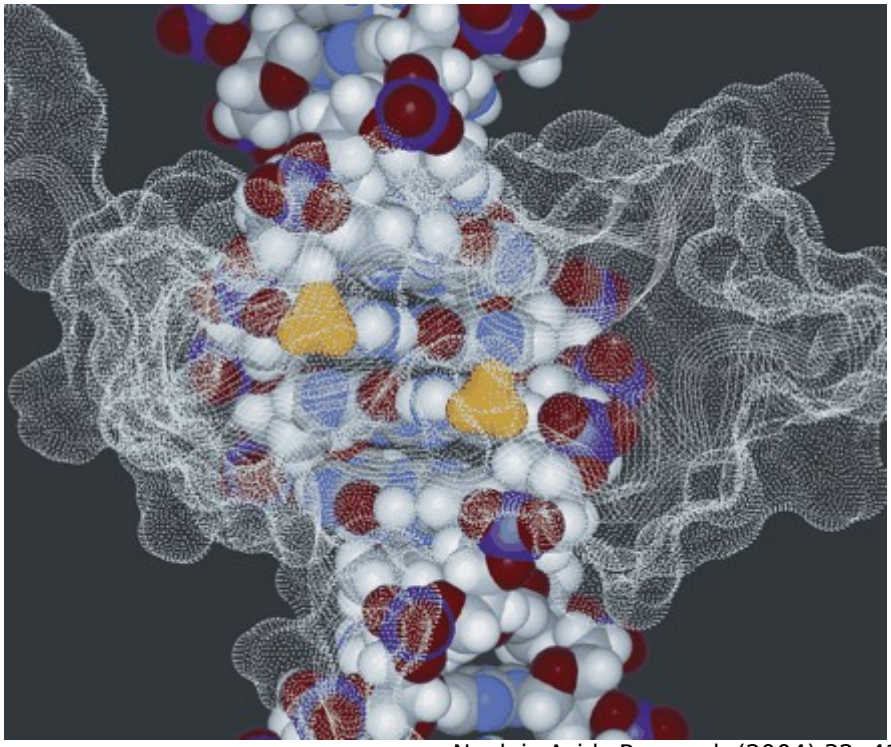
DNA methylation and gene activity in mammals



Nucleic Acids Research (2004) 32, 4100-4108

Miguel Constância

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Metabolic Research Laboratories, Department of Obstetrics & Gynaecology

University of Cambridge; email: jmasmc2@cam.ac.uk

Outline

- DNA methylation as an epigenetic mark
- What is the role of DNA methylation?
- Where is DNA methylation located in the genome?
- Are DNA methylation patterns dynamic or static?
- How is DNA methylation targeted?
- How is DNA methylation translated into a silencing signal?
- What are the future challenges?

5mC: The fifth base of DNA

- The draft version of the human genome, published in 200 heralded as the “book of life” – an ~3 billion “letter code” of 4 “letters” (A, T, G, C)

- Yet the book was “missing” 5-methyl cytosine – a modifie imparts an additional layer of heritable information upon t

- 5mC is commonly referred to as the fifth letter of the cod accounts for ~1%-6% of the nucleotides

- 5hydroxymethyl-cytosine (5hmC) was recently identified as the “6th letter” of the code (abundance and roles just now becoming known);

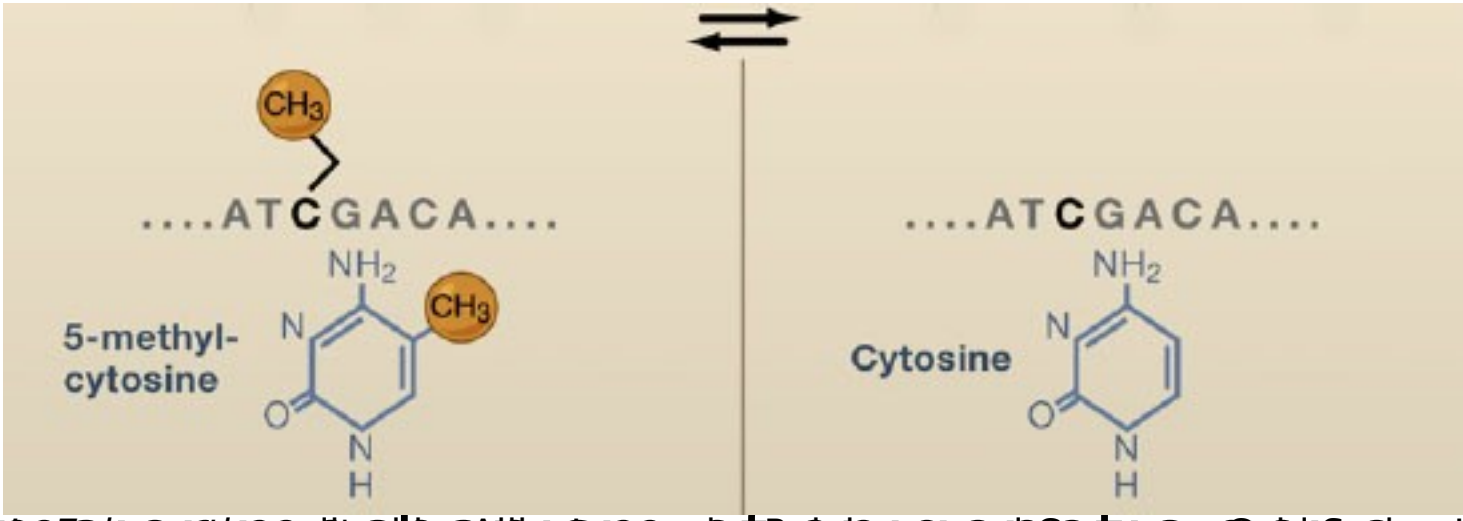
- And….5fc (5-formylcytosine) and 5caC (5-carboxylcytosine) are now the “7th” and the “8th”

DNA methylation

- DNA methylation evolved from prokaryotic restriction/modification systems
- It is present in major eukaryotic groups including plants, animals and fungi
- Evolutionarily volatile (lost in C.elegans; S. cerevisae, S.pombe)

Cytosine DNA methylation

- Covalent chemical modification of DNA catalyzed by DNA methyltransferases

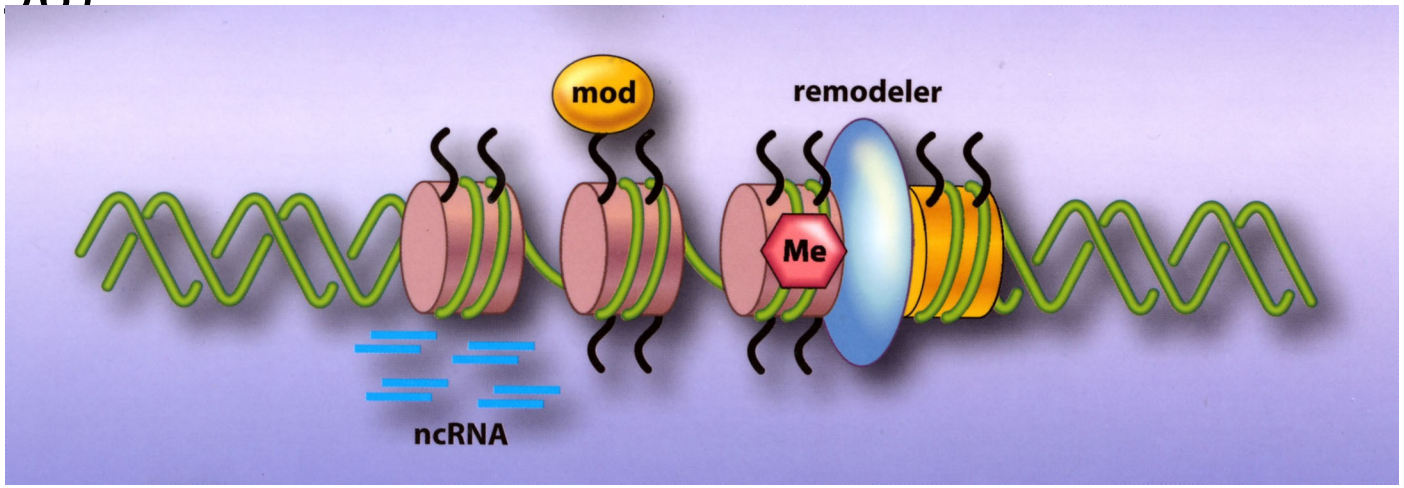


In Epigenetics edited by Allis, Jenuwein, Reinberg, and Caparros. Cold Spring HarborPres

- A major mechanism of epigenetic gene regulation

DNA methylation and Epigenetics

- Epigenetics: ‘study of mitotically and/or meiotically heritable changes in gene function that cannot be explained by changes in DNA sequence’ (Riggs et al, 1996)



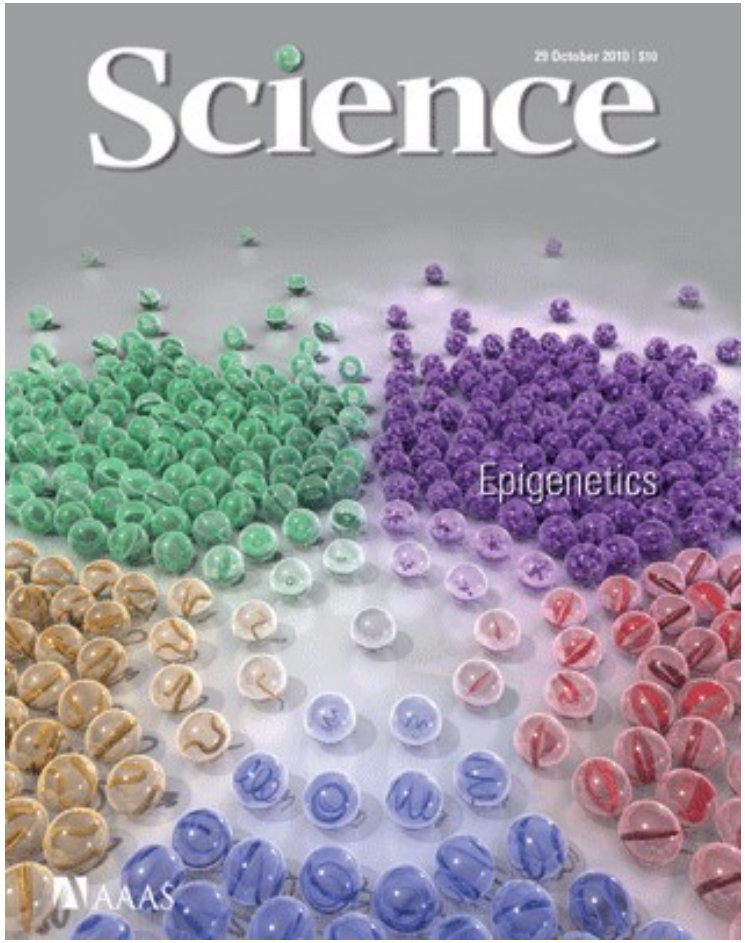
In Epigenetics edited by Allis, Jenuwein, Reinberg, and Caparros. Cold Spring HarborPress,

- DNA methylation patterns are maintained from cell to cell and even inherited from generation to generation
- DNA methylation of genes causes gene silencing (in most cases)

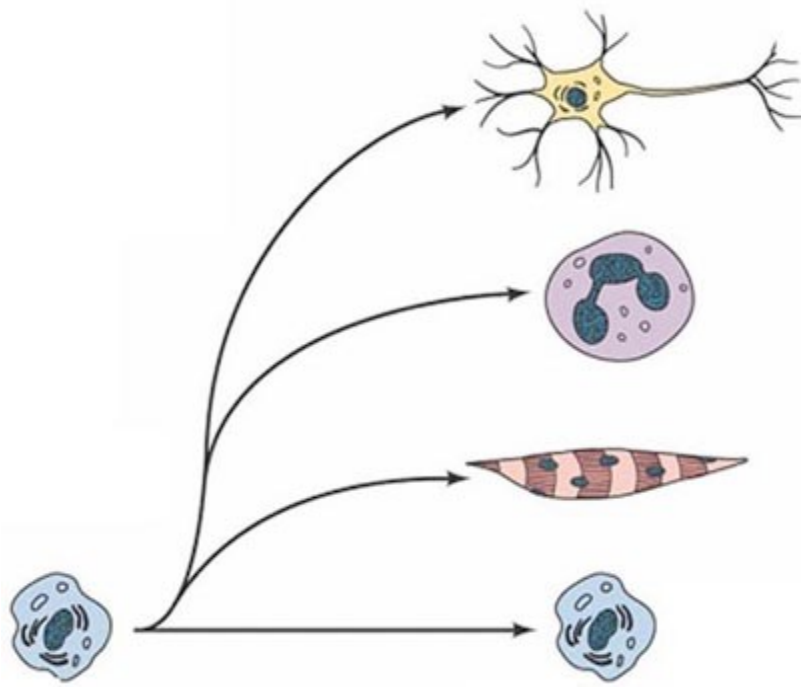
Epigenetic phenomena associated with DNA methylation

- Genomic stability and control of transposon activity
- Genomic imprinting in plants and mammals
- X-inactivation in mammals
- Cancer cell biology
- Transgene silencing
- Transcriptional regulation and long-term cellular memory

Development and differentiation (

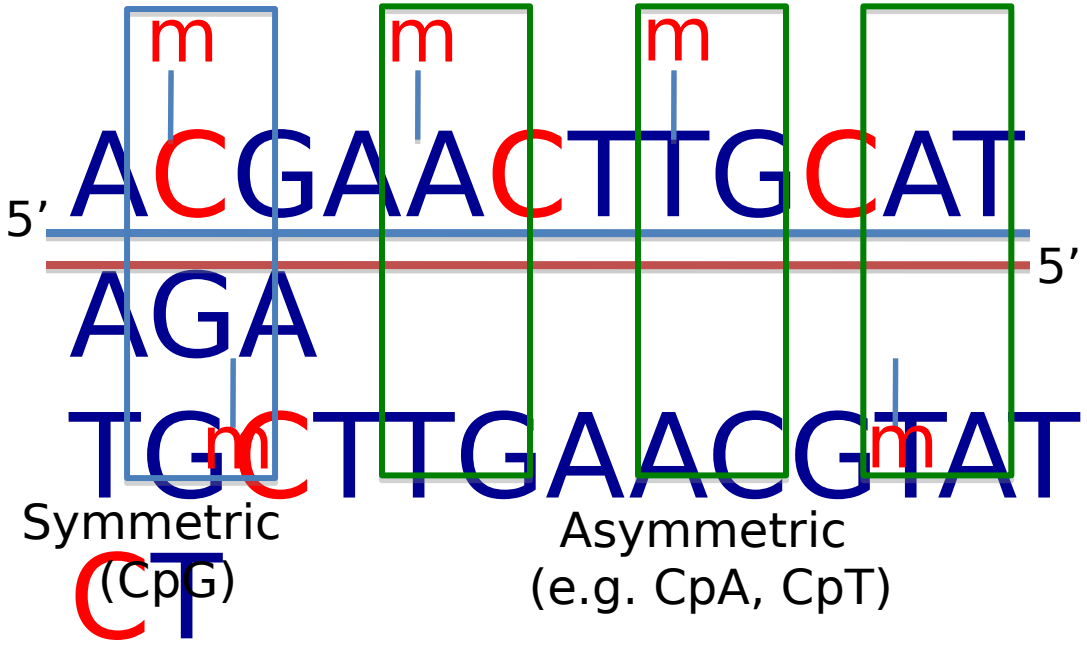


Multiple
Epigenomes



One
Genome

Methylated sequences in eukaryotic genomes



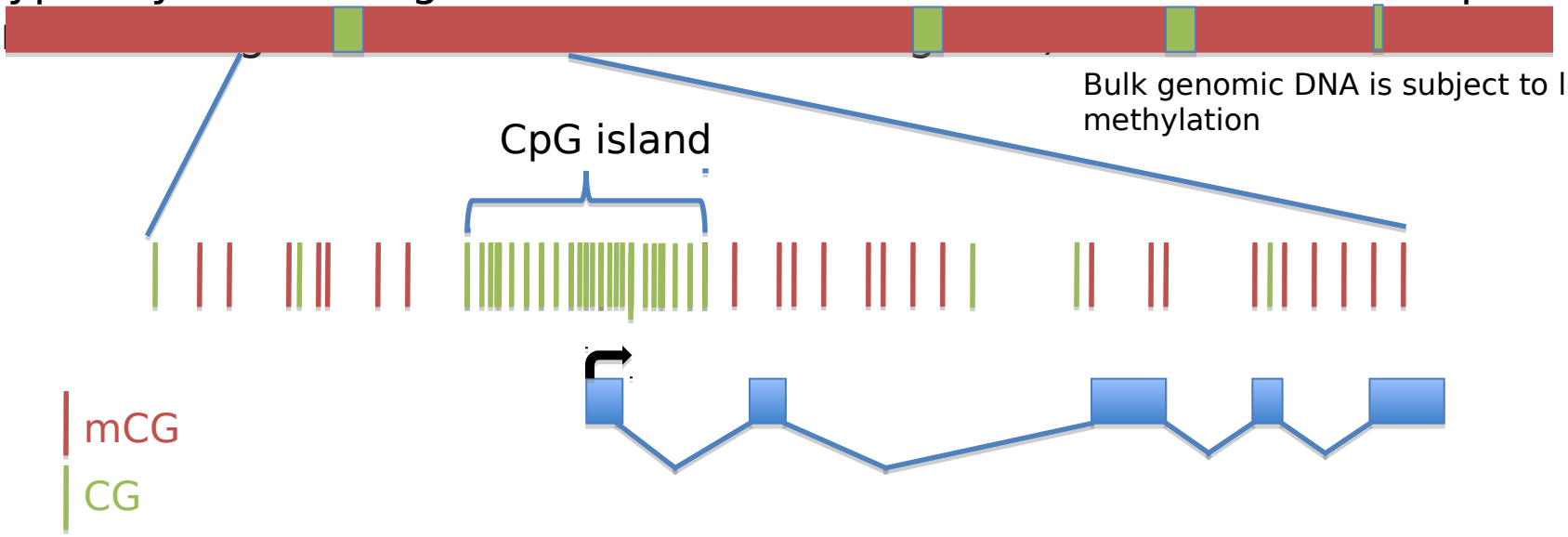
- CpG methylation (most abundant in mamma

hemimethylation serves as a guide for maintenance of patterns

- Non-CpG methylation (re-established *de nov*

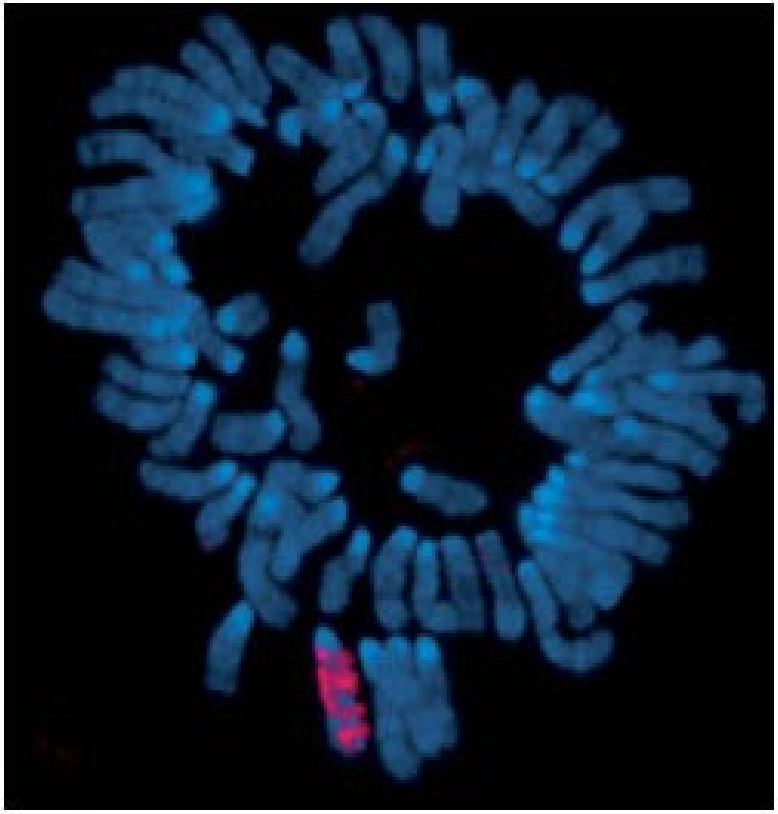
Where is DNA methylation located in the genome?

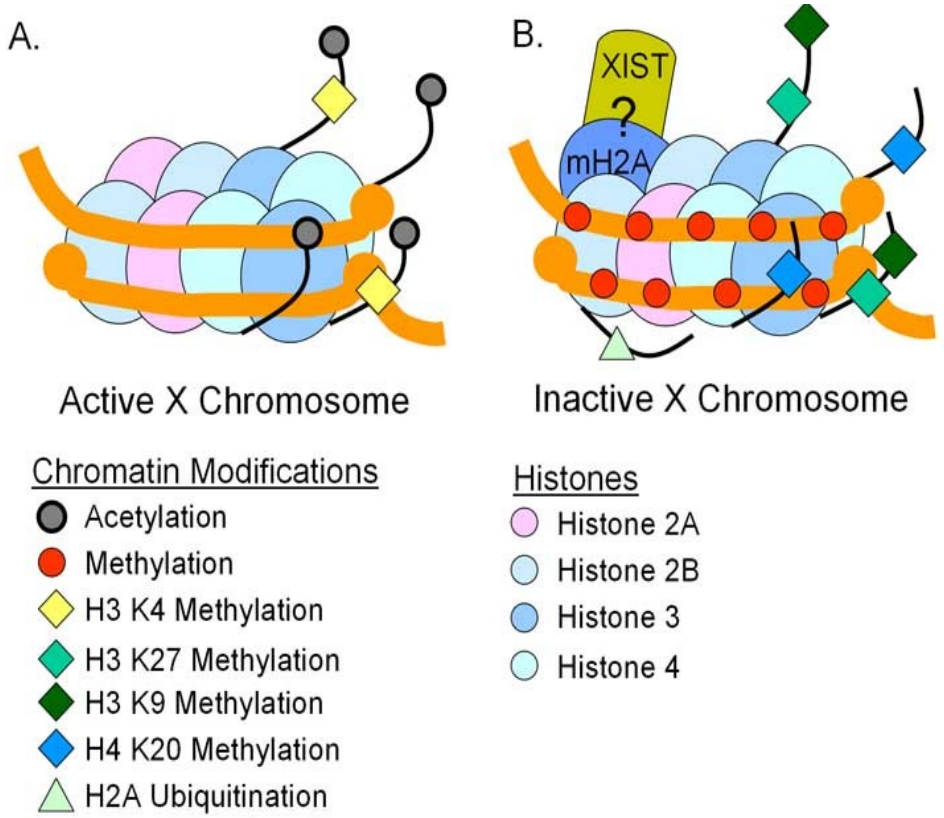
- In mammals, the majority of CpG pairs are methylated; DNA methylation is distributed throughout, including gene bodies, endogenous repeats and transposable elements
- 5mC spontaneously deaminates to thymine resulting in under representation of CpG (21% of that expected in the human genome)
- Genome at lower magnification (figure below): methylated sequences are punctuated by non-methylated sequences called CpG islands (typically 1kb in length with elevated G+C content and that overlap the p



Adapted from A.Bird, Henry Stewart talks @ http://hstal

Epigenetics in action: X-inactiv

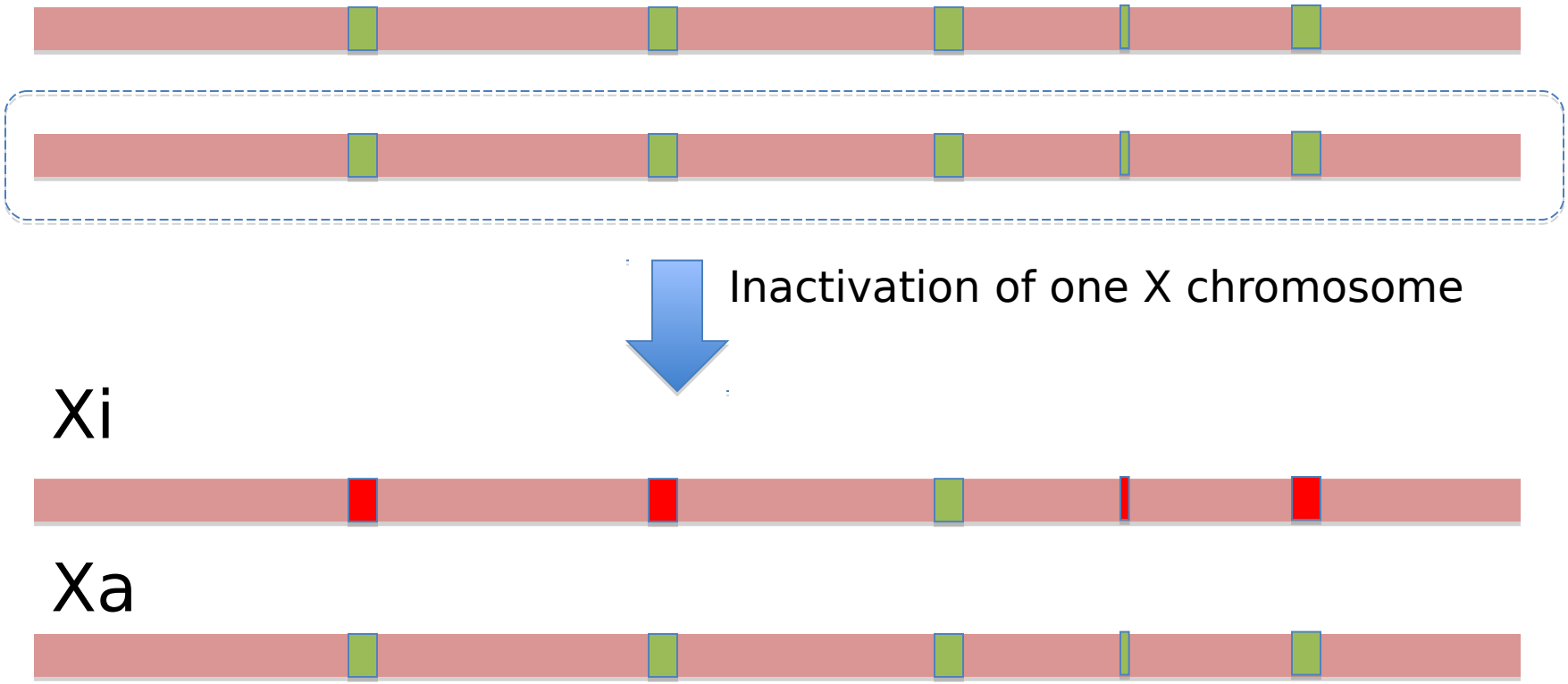




Ng K et al. (2007) *Embo Reports* 8:35

Chang et al. (2006) *Front Biosc* 11:852

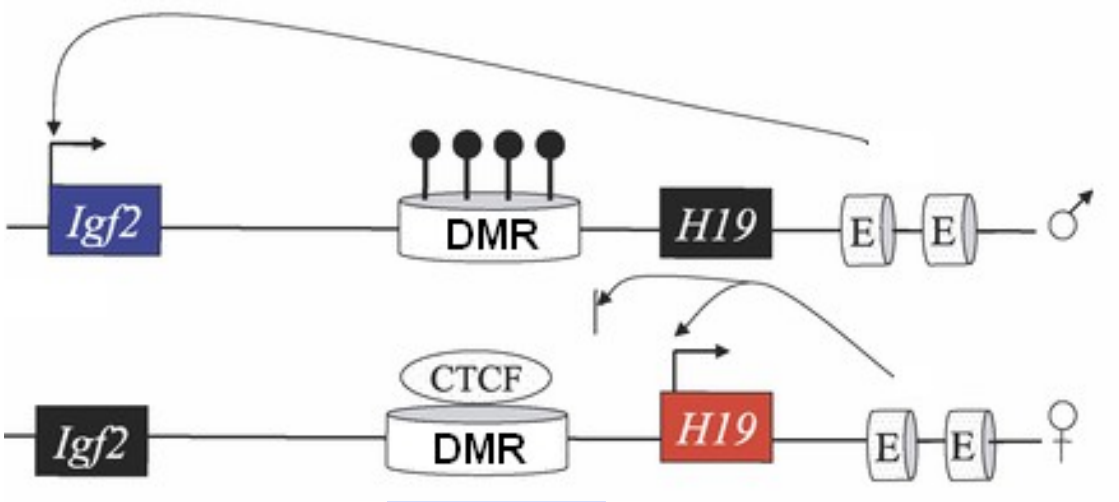
De novo methylation at CpG islands on the inactive X chromosome



From A.Bird, Henry Stewart talks @ http://hstalk

Regulation of imprinting clusters through epigenetic regulation of an insulator

- IGF2 is a paternally expressed gene (growth factor)
- H19 is a maternally expressed gene (non-coding RNA)



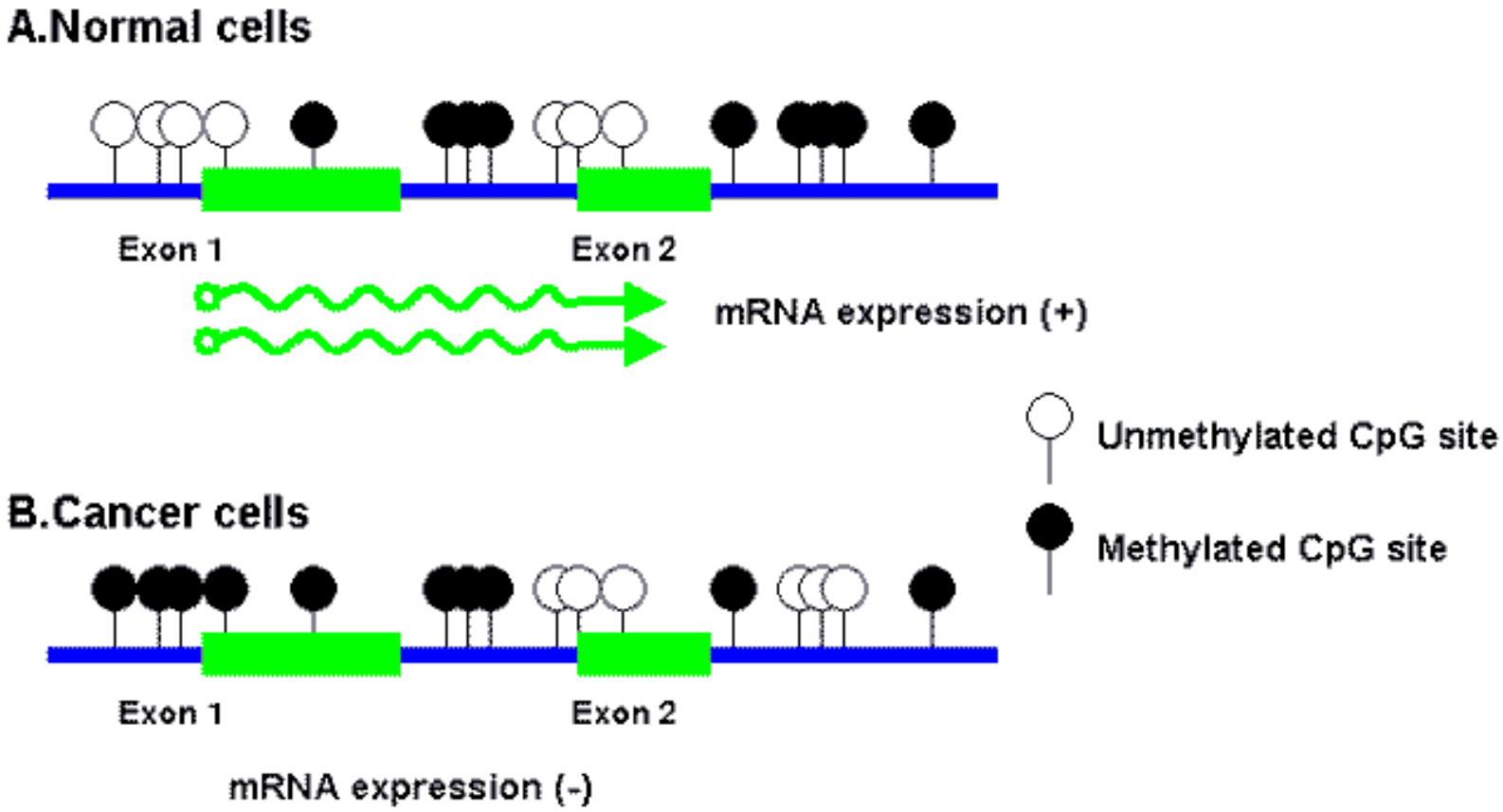
ICR1*

Boundary and enhancer access model

E-Enhancer
DMR-Differentially methylated re
CTCF-Insulator protein

Bell & Felsenfeld 2000 *Nature* **405**:482

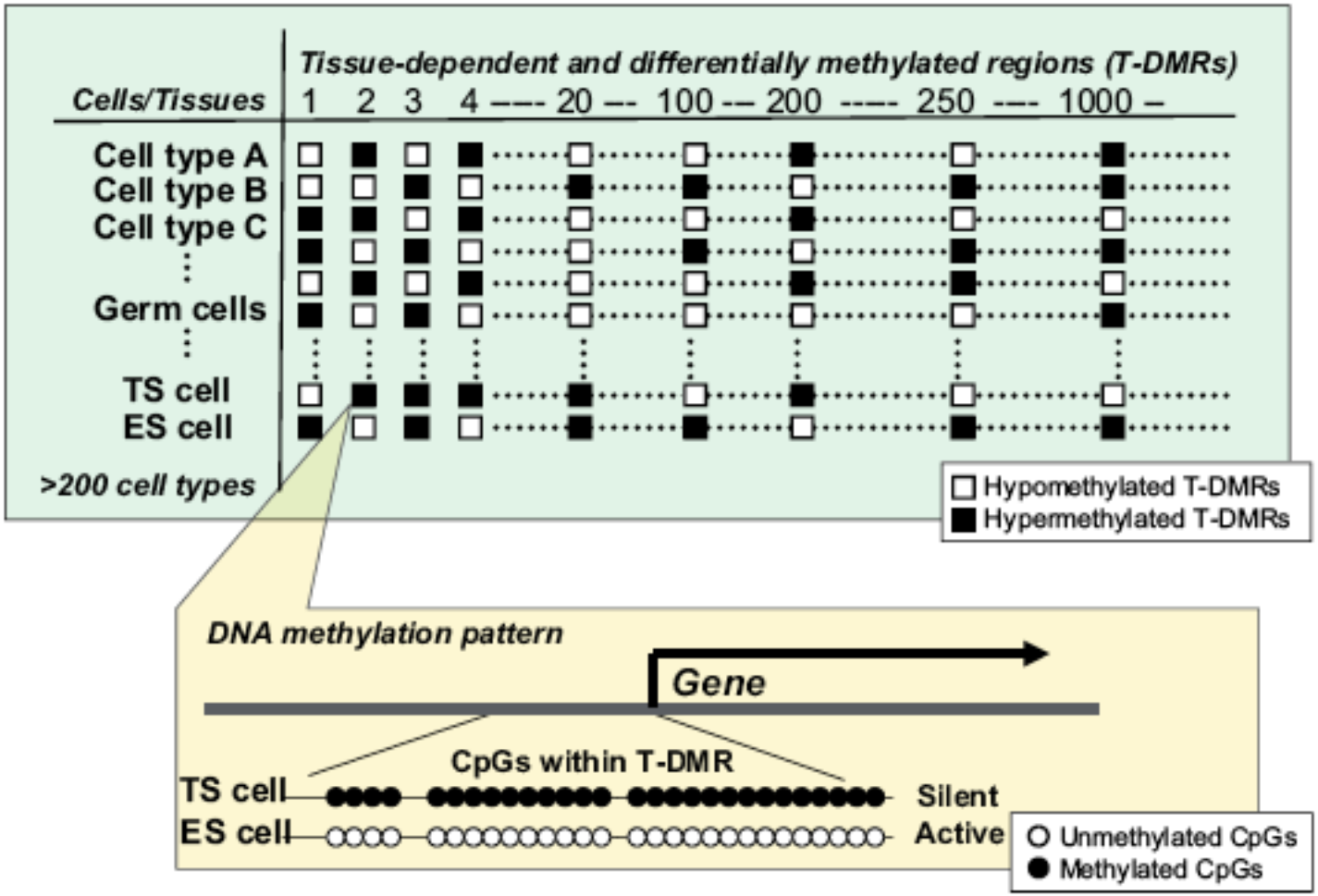
Hypermethylation of tumor suppressor genes



www.ncc.go.jp/en/nccri/divisions/14carc/14carc0

Cell-type specific DNA methylati

(Differentially methylated T-DMRs)

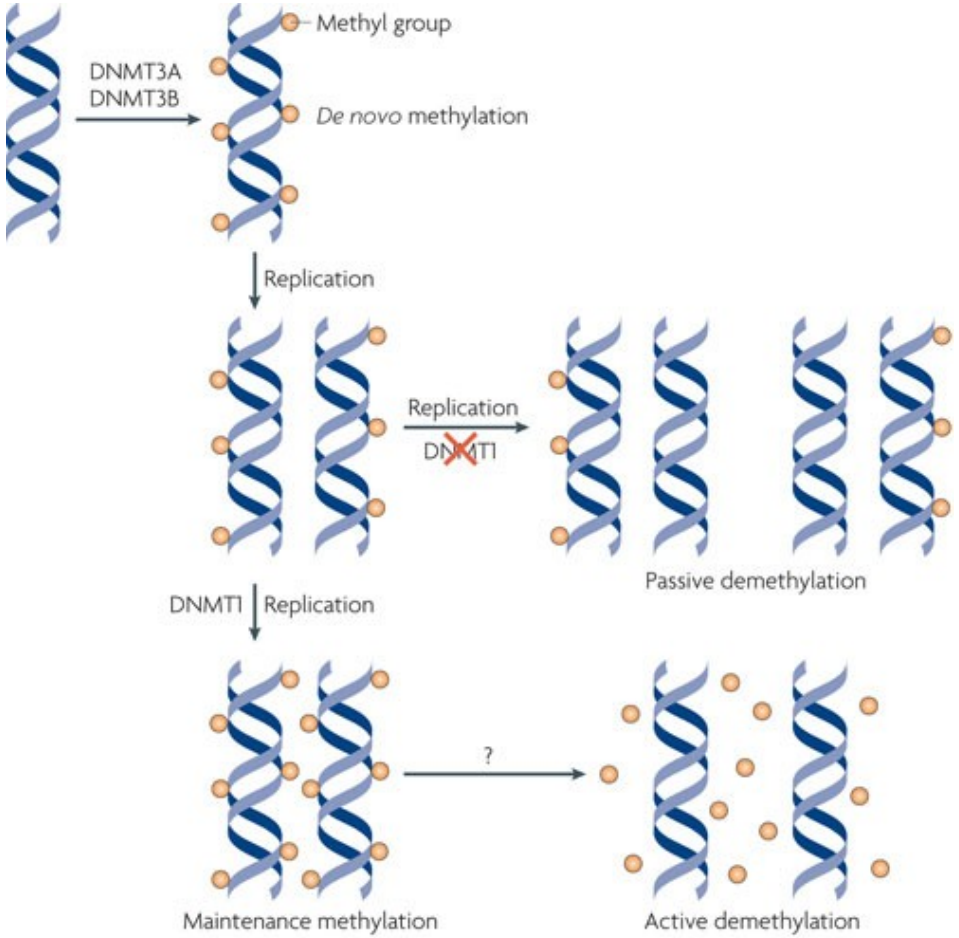


- Tissue specific methylation is observed at genes essential for development, suggesting a programmed mechanism of DNA methylation?

Ikegami *et al.* (2009) *Int J Dev Biol* **53**

Is DNA methylation dynamic, static, or a bit of both?

CpG methylation in mammals

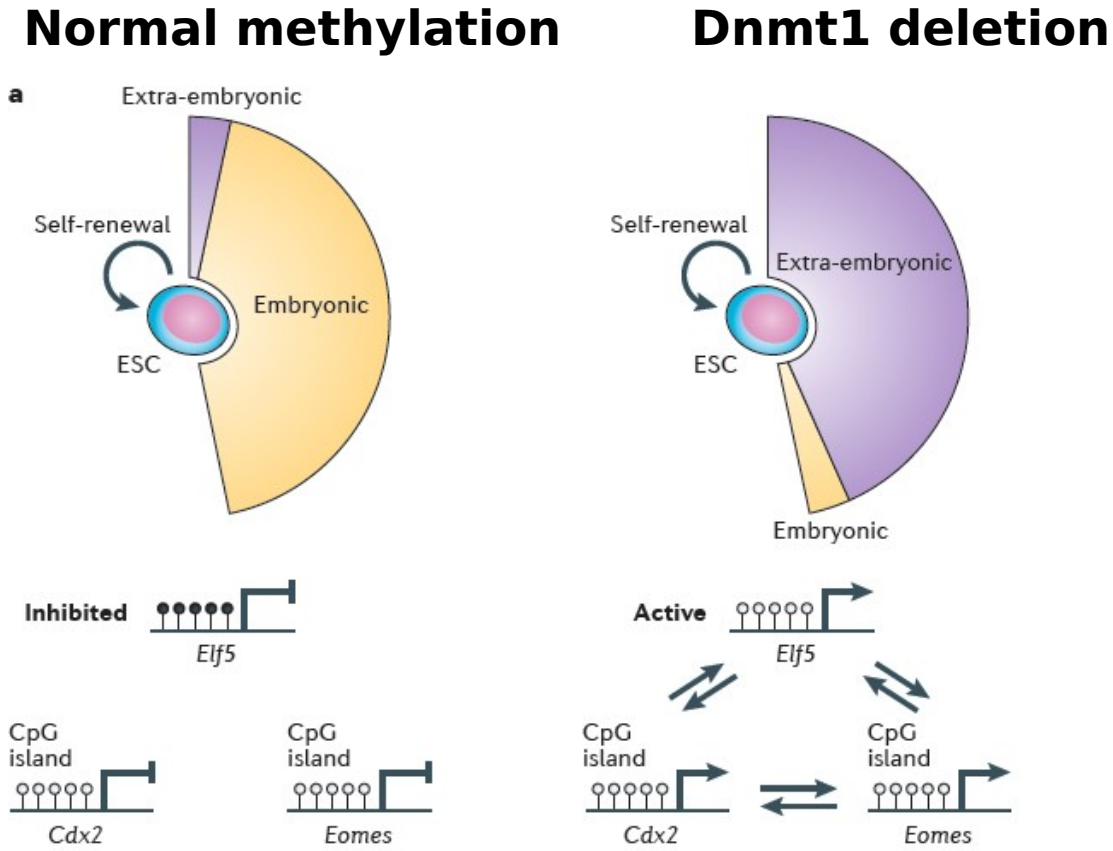


Wu S and Yi Z *2010, 11:607-620*

Nature Reviews | Molecular Cell Biology

- DNA methylation is established by *de novo* methyltransferases
- Symmetric methylation patterns are maintained after replication by maintena

Lineage restriction and renewal of embryonic stem cells

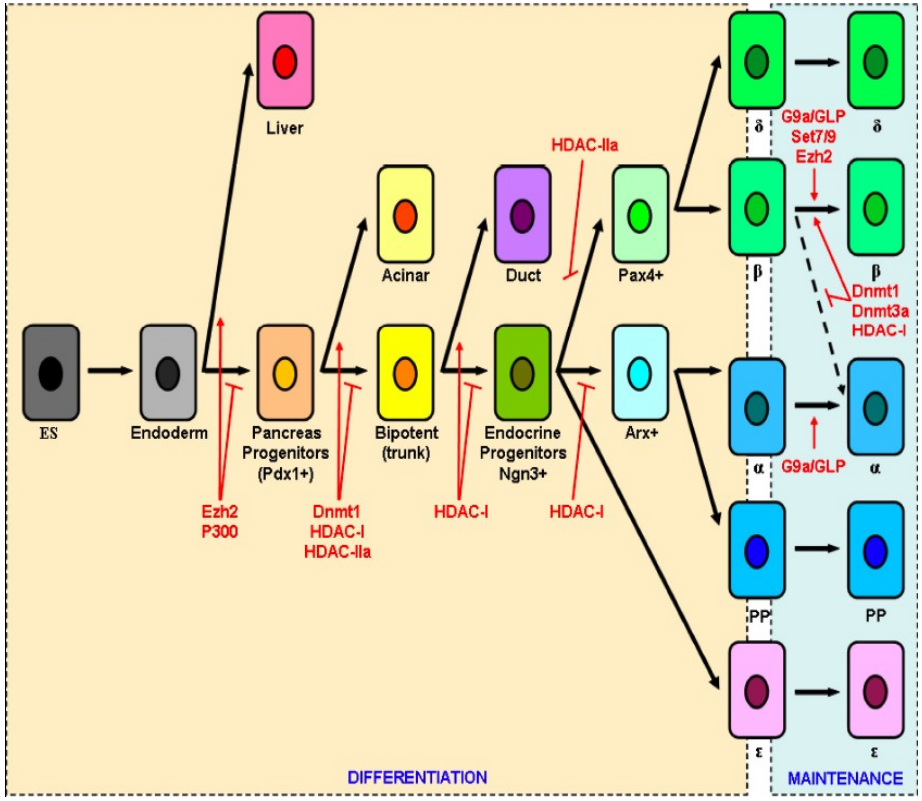


• Maintenance of embryonic potential is in part conferred by hyperr
and silencing of trophoectodermal TF Elf5

Smith & Meissner *2013, Nat Rev Genet 14:20*

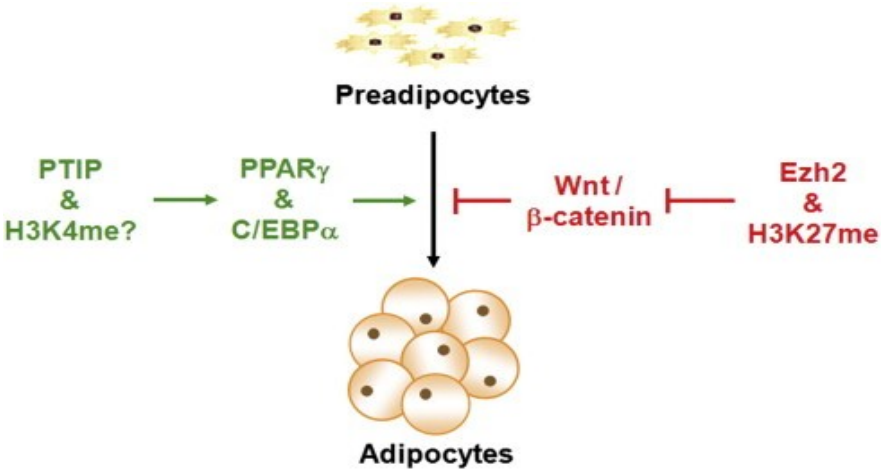
Epigenetic marks contribute to cell lineage determination

Epigenetic regulation of pancreatic development



Cell Mol Life Sci. 2013 May;70(9):1575-95.

Epigenetic regulation of adipoge

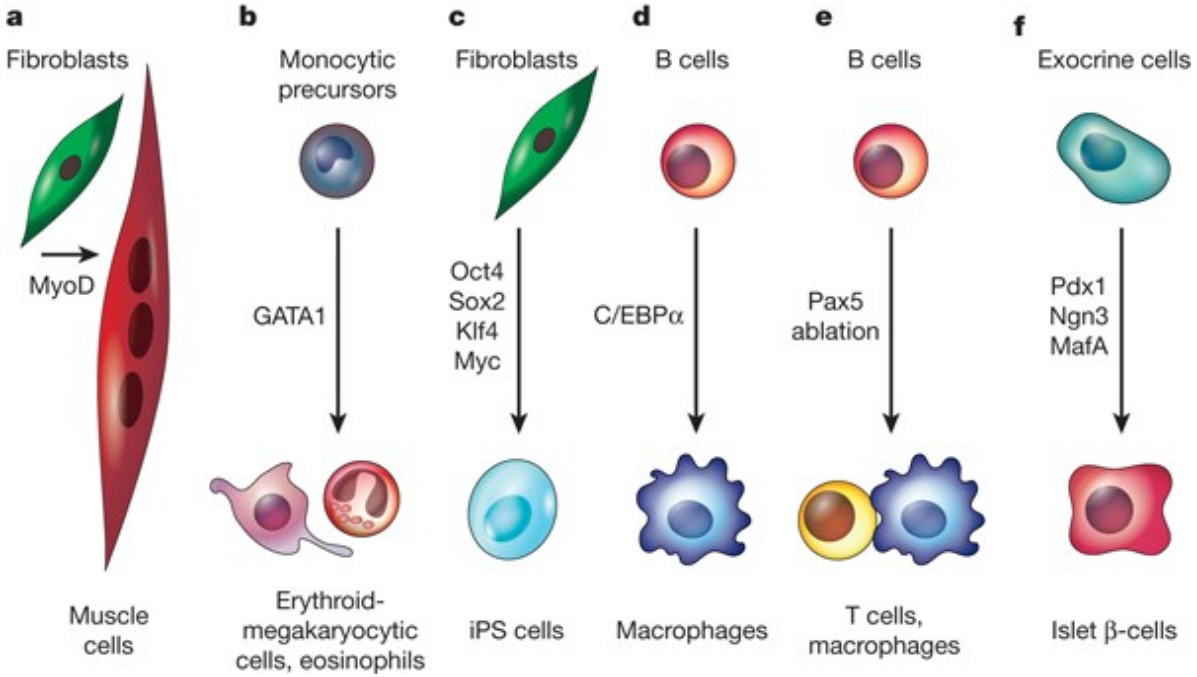


Biochimica et Biophysica Acta (BBA) – Issue 7 2012 727 - 732

How are differentiated cells “reprogrammed” to stem cells?

Does this require epigenetic reprogramming?

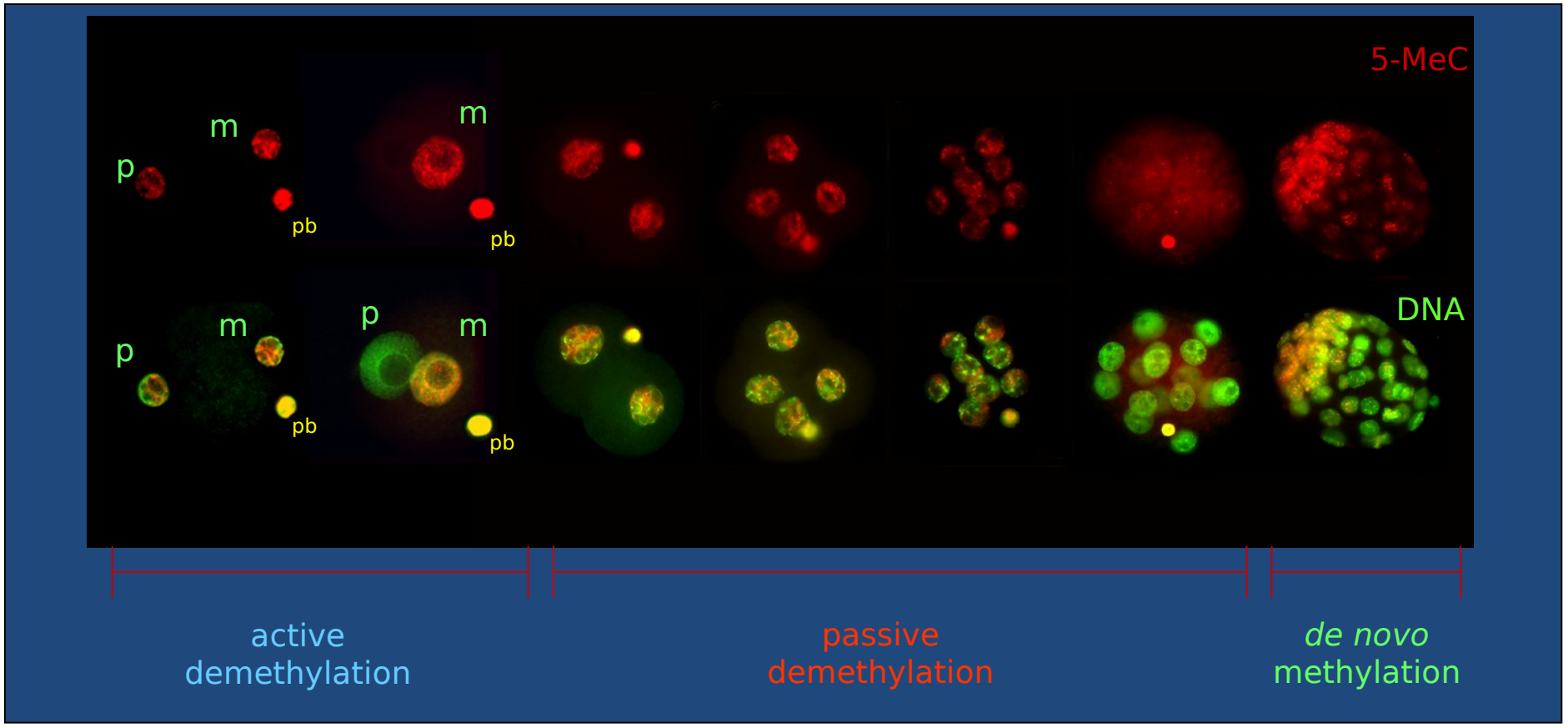
• Examples of transcription factor overexpression or ablation experiments that result in cell fate changes



Thomas Graf & Tariq Enver *Nature* **462**, 587-594 (2009) doi:10.1038/nature08533

nature

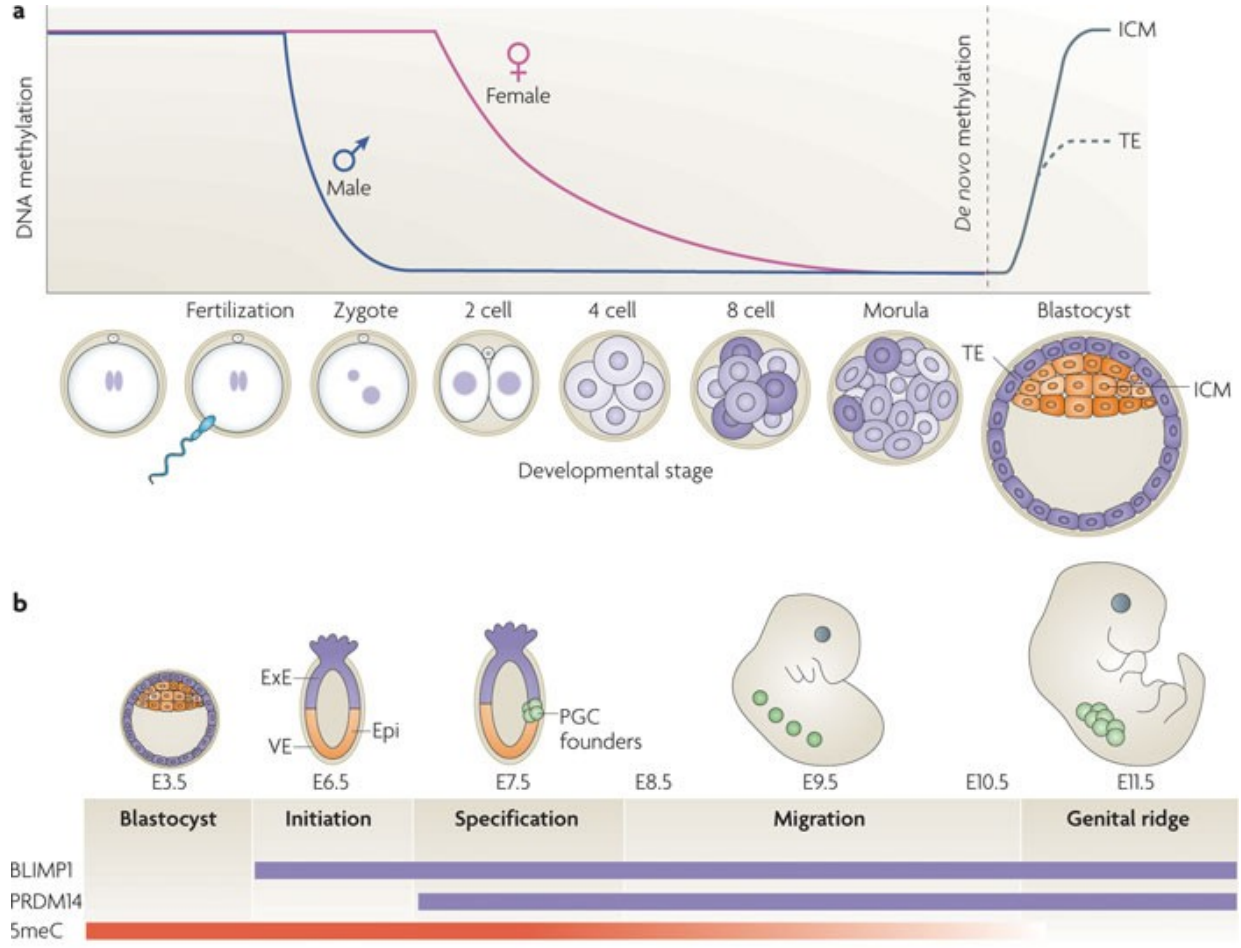
Active and passive demethylation in t



Slide courtesy of F. Santos & W. Dean, The Babrahar

• The rapid loss of DNA methylation that occurs within the period of a single ce
the presence of enzymes that actively remove 5-methylcytosine
• Dnmt1 is excluded from the nucleus from the 1 to 8 cell stages, leading to pas

Active and passive demethylation in t

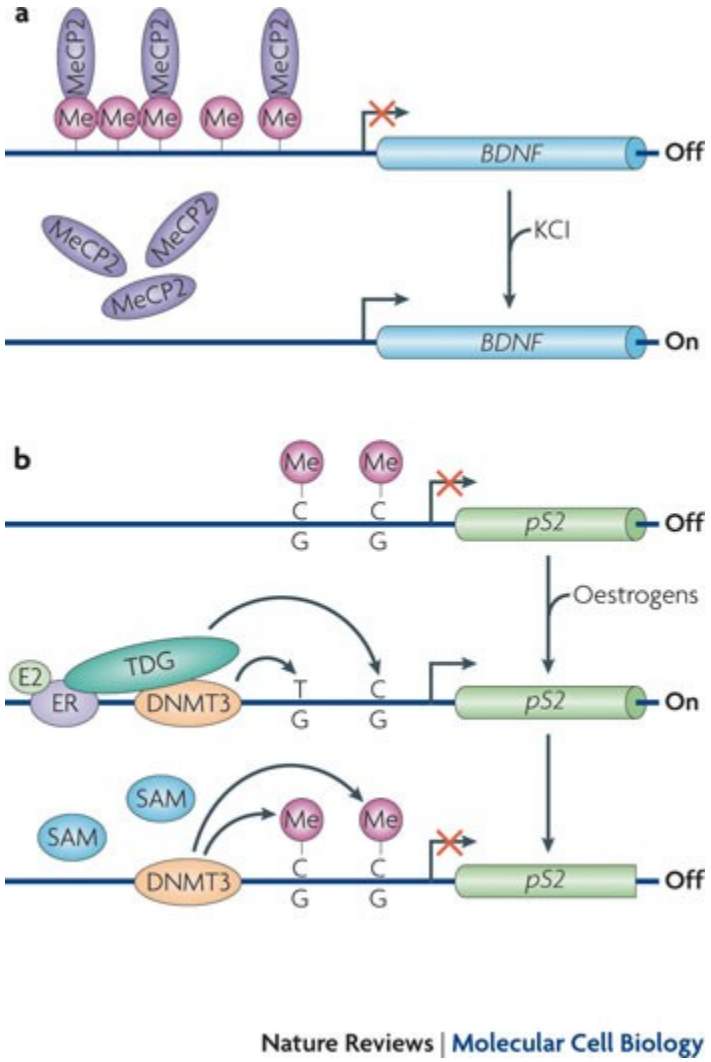


Wu S and Yi Z *2010, 11:607-620*

Nature Reviews | Molecular Cell Biology

- **DNA is demethylated genome-wide at two points during mamalian development**
- **Erasure of methylation and other chromatin marks might be**

Gene-specific active DNA demethylation in somatic cells



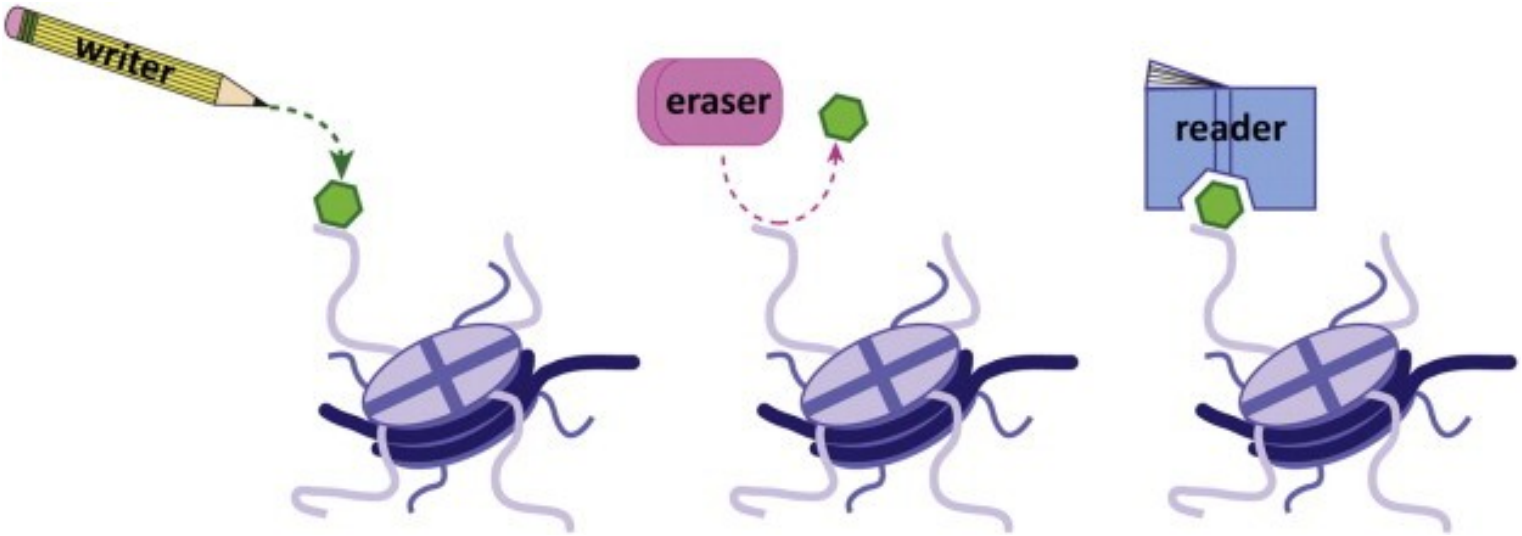
Post-mitotic neurons

Deamination of 5mC

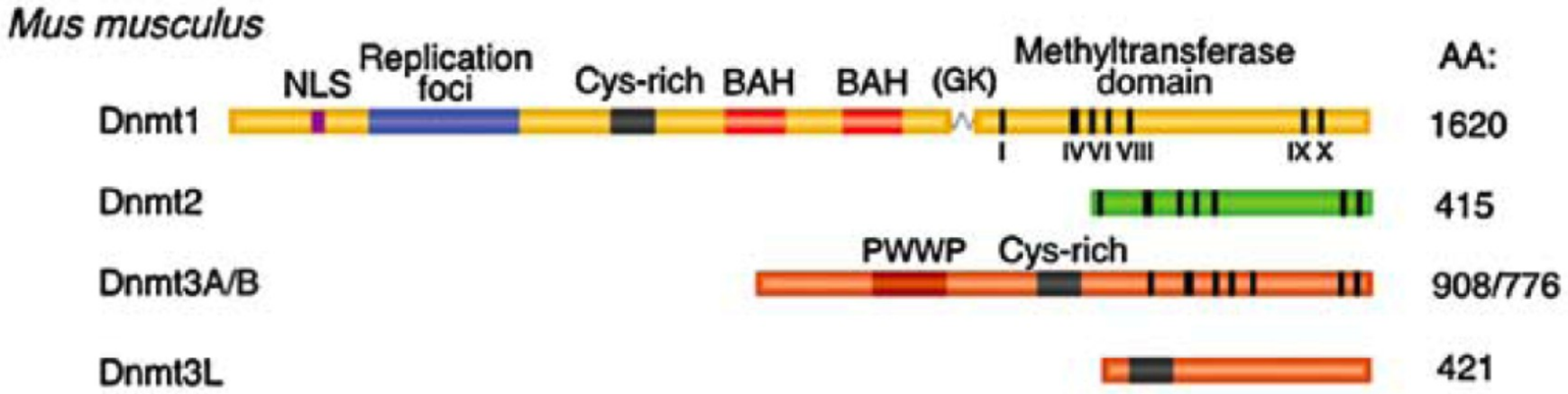
Cyclical rounds of methylation and demethylation

Wu S and Yi Z *2010,*

‘Writers’, ‘Readers’ and ‘Erasers’ of CpG methylation



DNA methylation is carried out by DNA methyltransferases

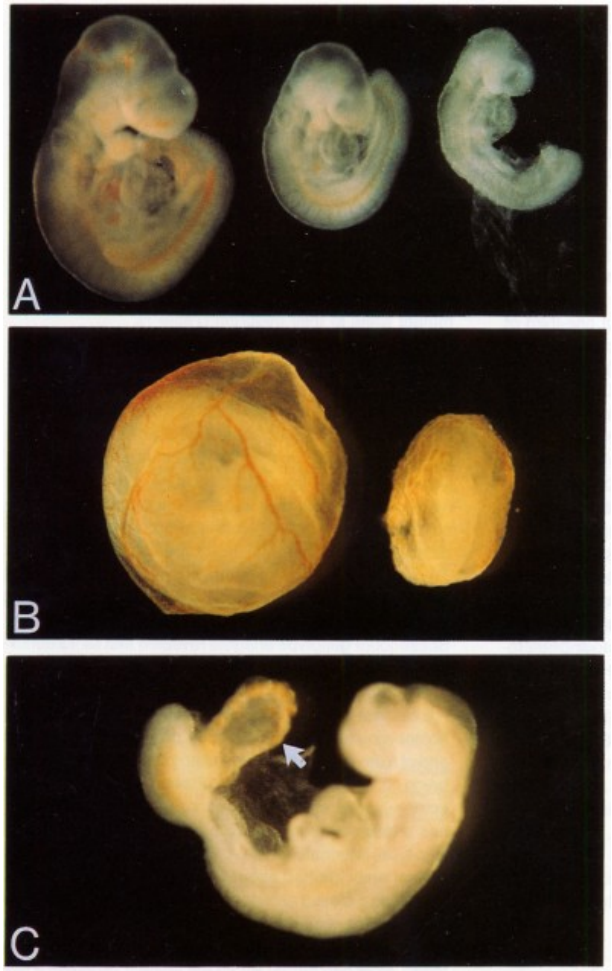


- Dnmt1 is the maintenance methyltransferase; prefers hemimethylated Cp
- Dnmt2 methylates a small RNA ($tRNA^{Asp}$)
- Dnmt3 A/B are required for *de novo* methylation

- Dnmt3L lacks active methyltransferase activity; it functions as a regulator and Dnmt3b; Essential for establishment of maternal imprints in growing oo establishment of methylation at retrotransposons in non-dividing prosperm

Goll & Bestor, *Annu Rev Biochem* (2005) **7**
Ooi *et al.* (2009) *J Cell Sci* **122**:287
Jones & Liang (2009) *Nat Rev Genet* **10:**8

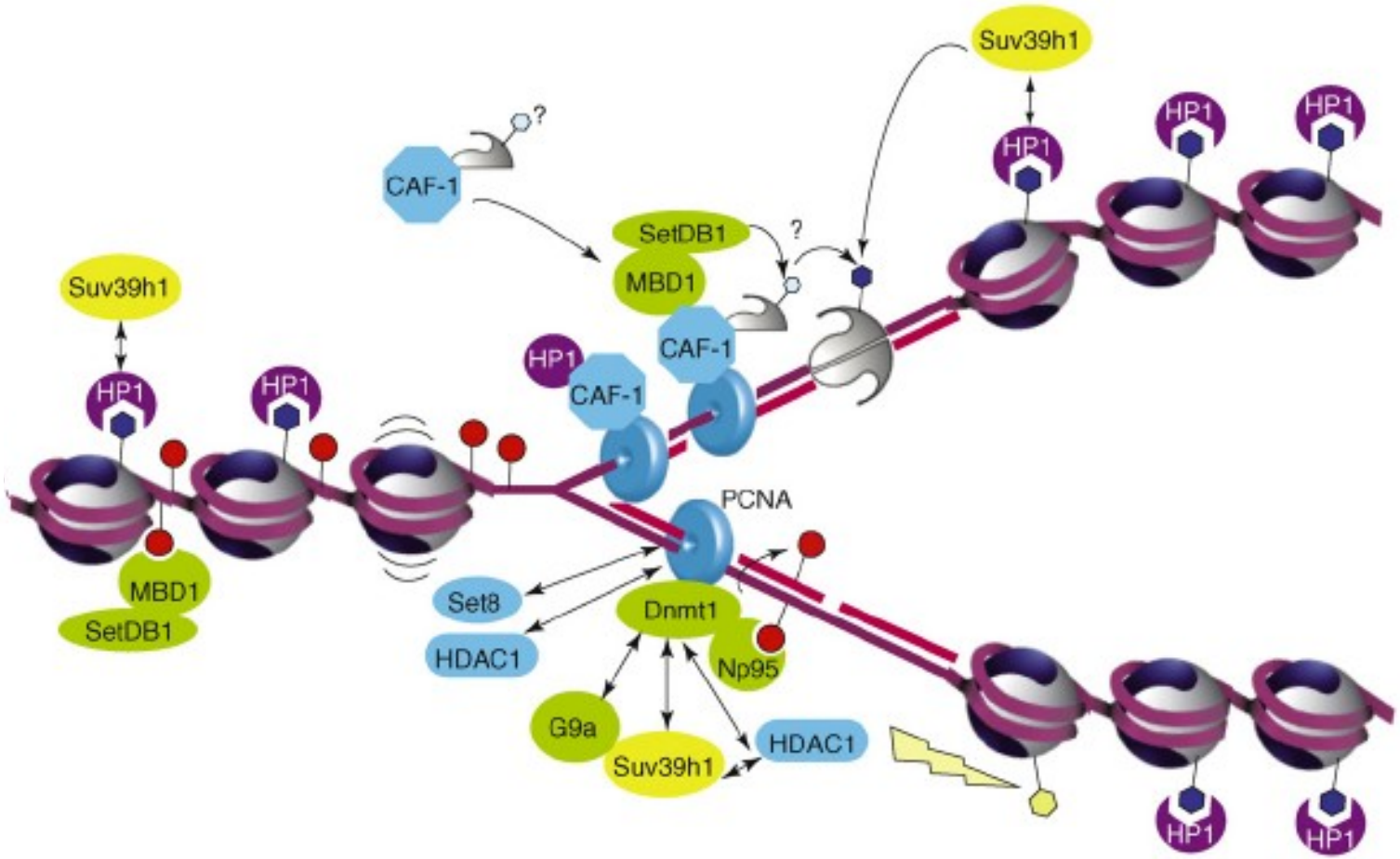
Dnmt1 knock-out die during embryogenesis



- Reduction of CG methylation to 5-30% of wild-type; retrotransposon expression is reactivated
- Defects in X-inactivation and genomic imprinting

Li *et al.* (1992) *Cell* **69:**915; Li *et al.* (1993) *Nature*

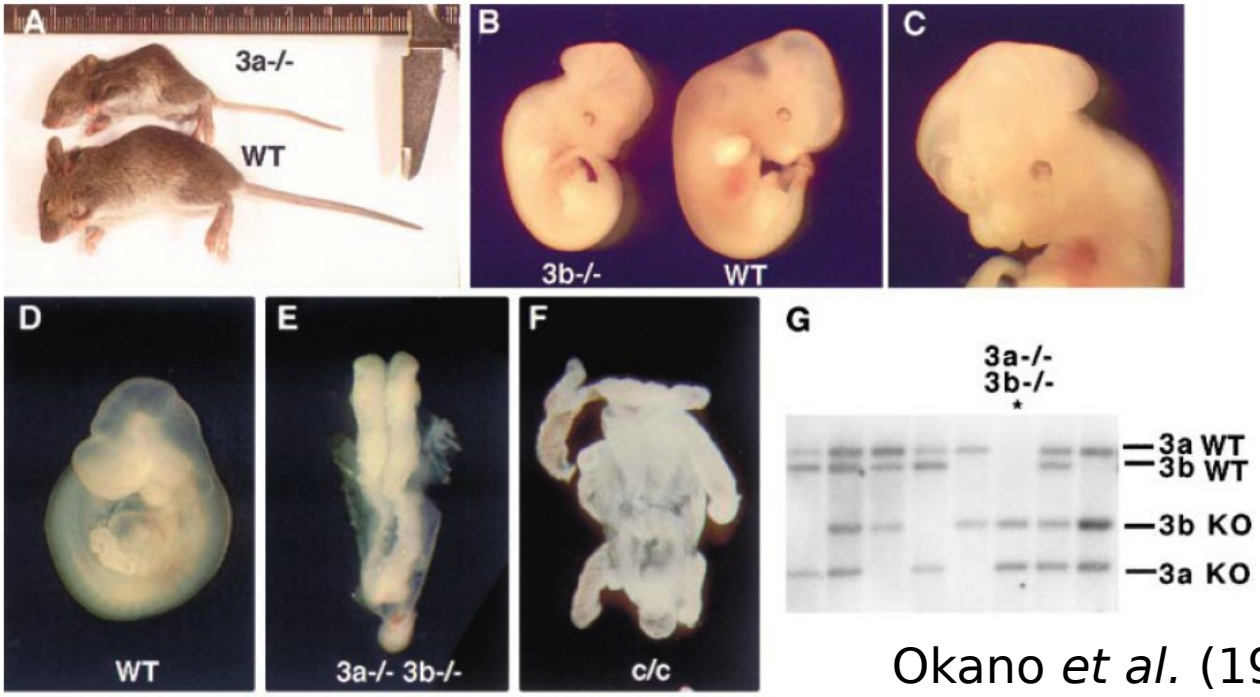
Dnmt1 and the heritability of epige



PCNA together with Np95 (also known as Uhrf1) recruits Dnmt1 which methy
hemimethylated CpG sites on daughter strands. Np95 has recently been sho
essential for maintaining DNA methylation.

Probst *et al*. (2009) *Nat Rev Mol Cell Biol* **10**

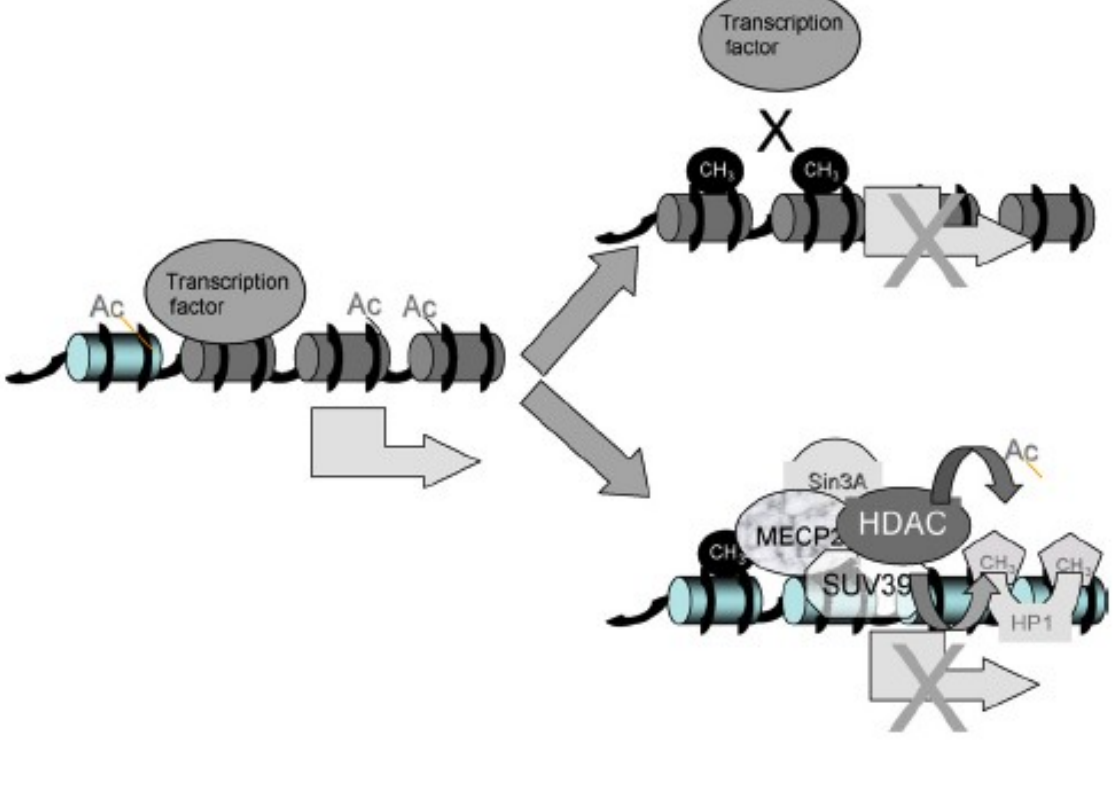
Dnmt3a/Dnmt3b double mutant knock-out mice



Okano *et al.* (1999) *Cell* **99:**24

- Double mutants die during embryogenesis
- Genome wide losses of DNA methylation
- Point mutations in Dnmt3b cause the human disease IC syndrome

How is methylated DNA translated into a silencing signal?



1. Repulsion of transcription factors

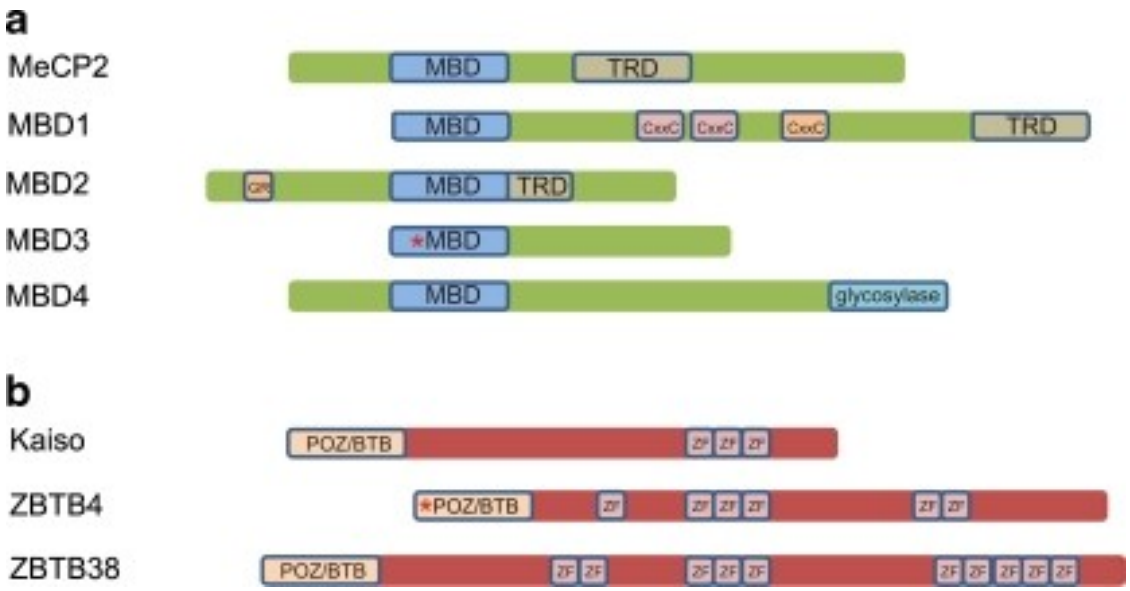
2. Attraction of Repressors/repulsio of activators

Sasai & Defossez (2009) *Int J Dev Biol* **53**:323; Bogdanovic & Veenstra (2009) *Chron*

The methyl-CpG binding protein family

MBD family

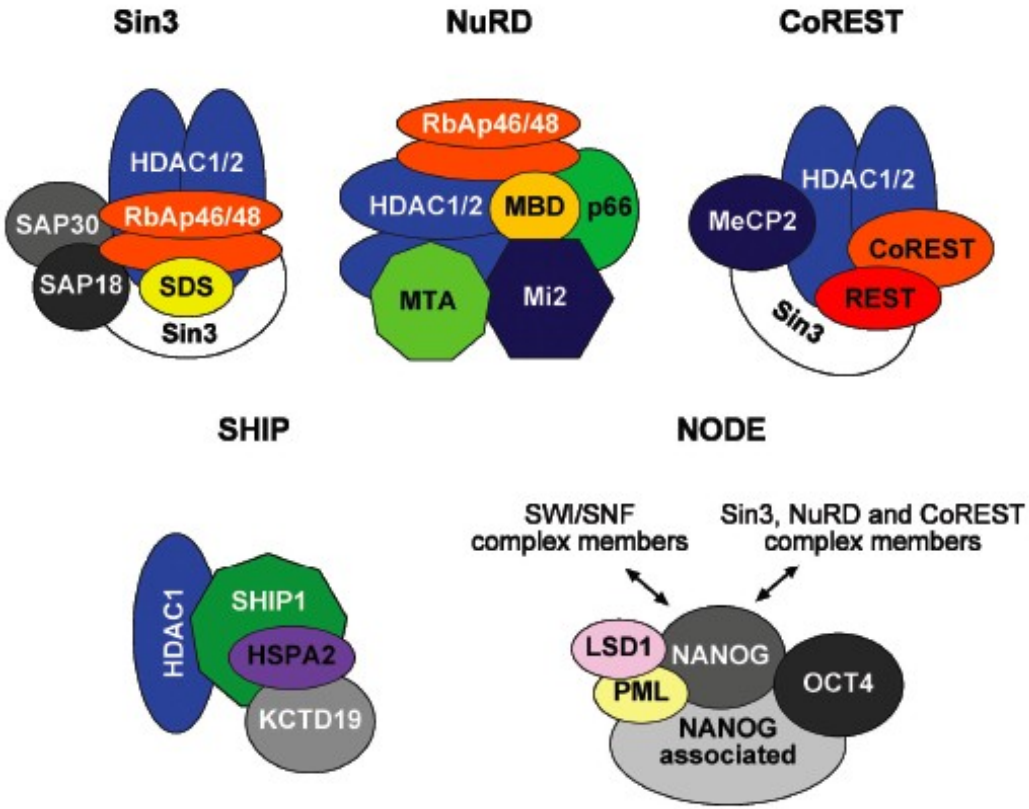
Kaiso family



- Mutations in MeCP2 are the cause of Rett Syndro

Sasai & Defossez (2009) *Int J Dev Biol* **53**:323; Bogdanovic & Veenstra (2009) *Chron*

MBD's as members of multiprotein complexes function in transcriptional repression



Co-REST: repression of neural genes

NuRD: MBD2, MBD3 and transcriptional repression

- These proteins are thought to associate with histone deacetylase ac establish silent chromatin; other interactions result in transcriptional

Sasai & Defossez (2009) *Int J Dev Biol* **53**:323; Bogdanovic & Veenstra (2009) *Chromo*

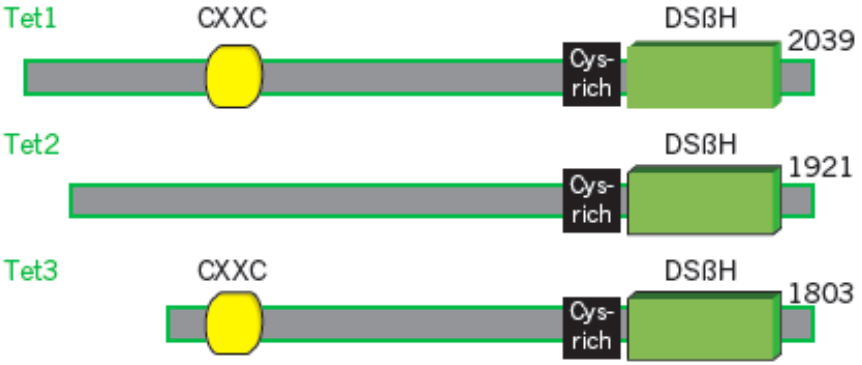
Active demethylation: many roads lead to Rome...

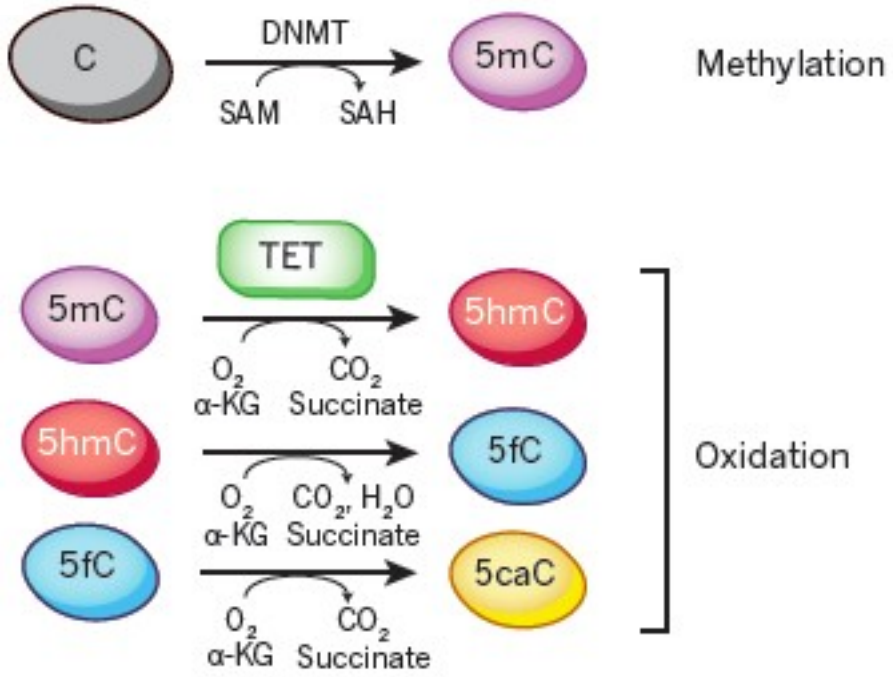
- Multiple mechanisms exist to carry out active DNA demethylation (use of each one is dictated by specific biological context)

  - Enzymatic removal of the methyl group of 5mC
  - Base excision repair (BER) through direct excision of 5mC
  - Deamination of 5mC to T followed by BER of the T-G mismatch
  - nucleotide excision repair (NER)
  - oxidative demethylation
  - radical S-adenosylmethionine (SAM) based de-methylation

Wu S and Yi Z *2010 Nat Rev Mol Cell Biol 11*

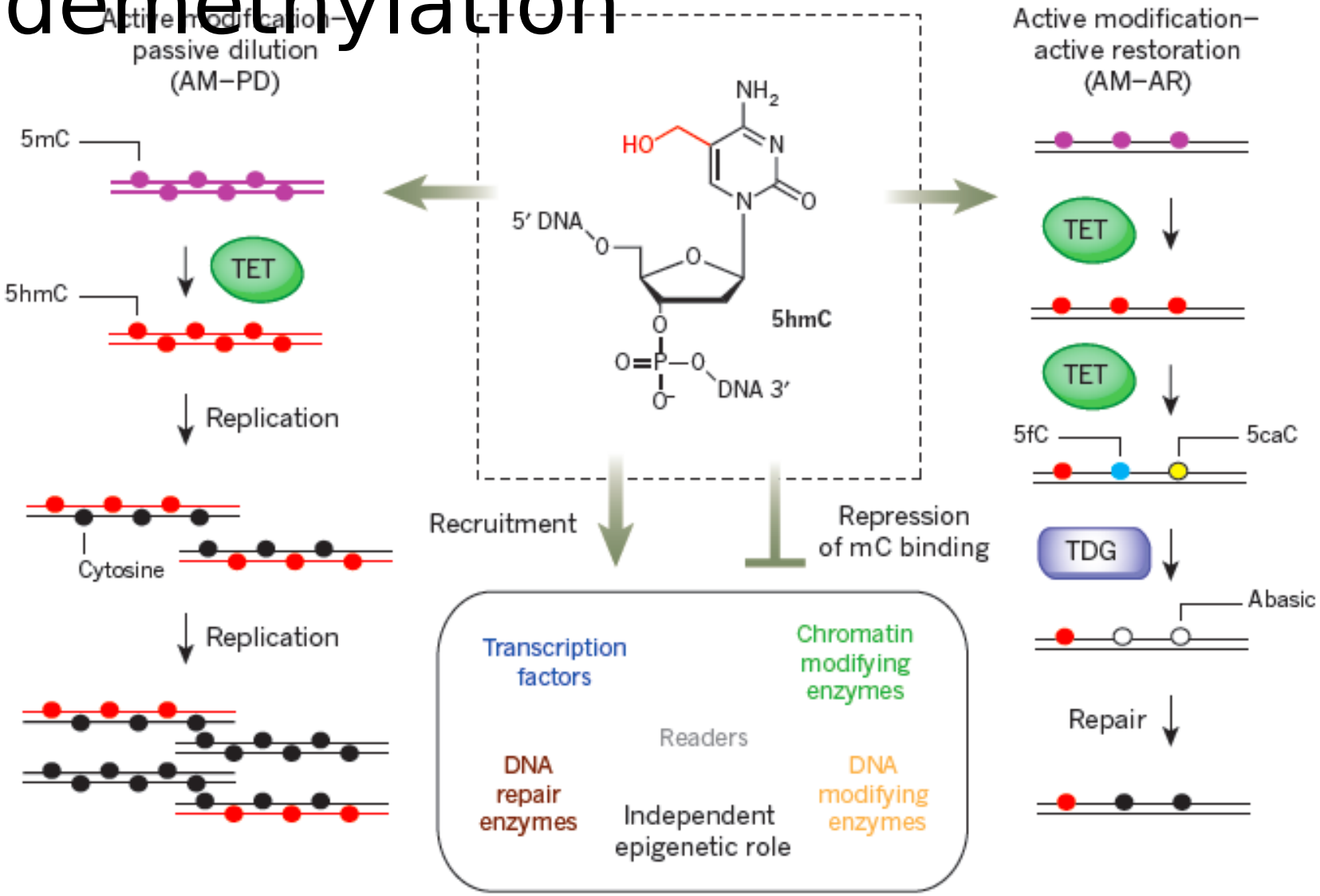
TET function in oxidation of modified C bases





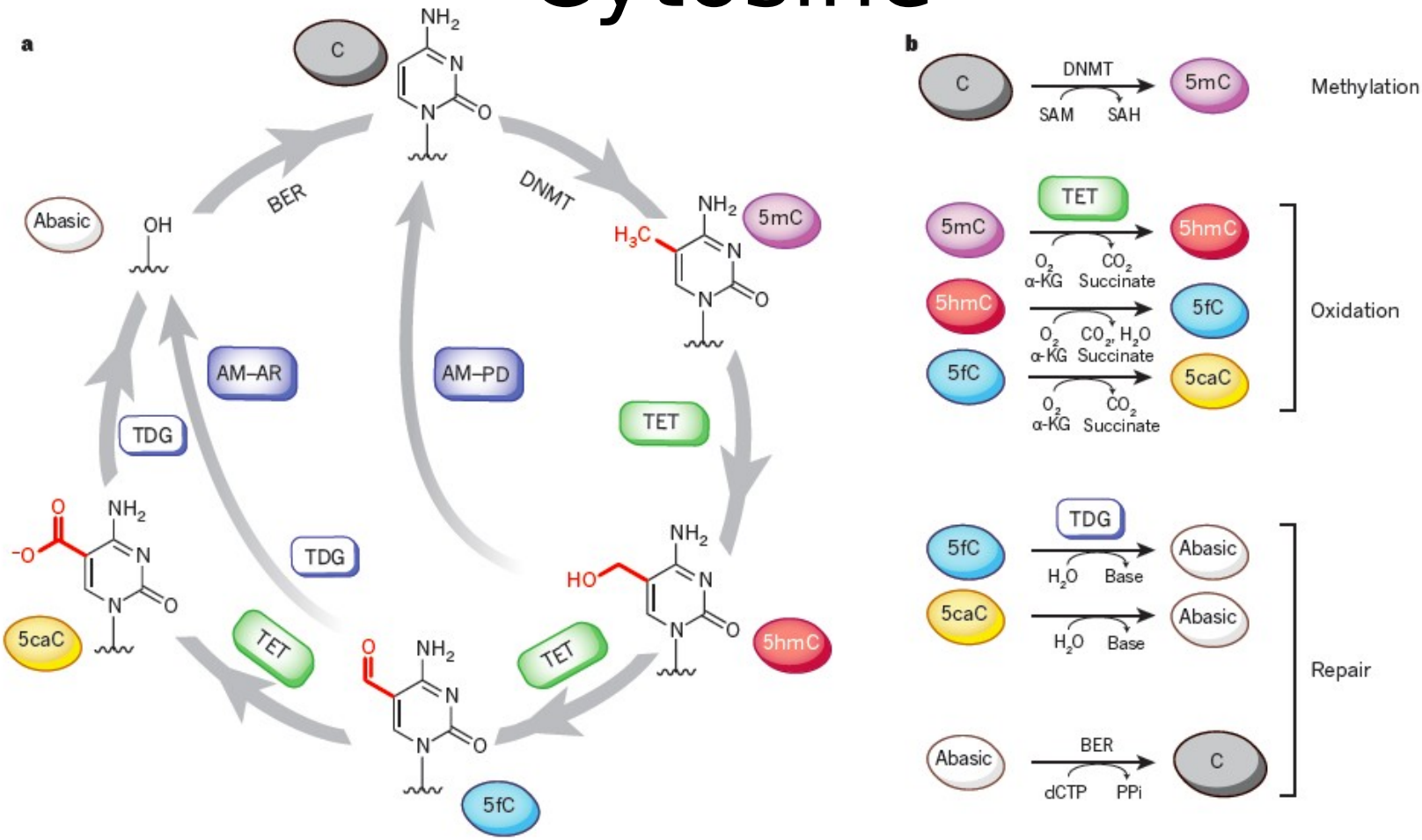
Kohli & Zhang 2013, *Nature 502:4*

Roles of 5hmC in DNA demethylation



Kohli & Zhang 2013, *Nature 502:4*

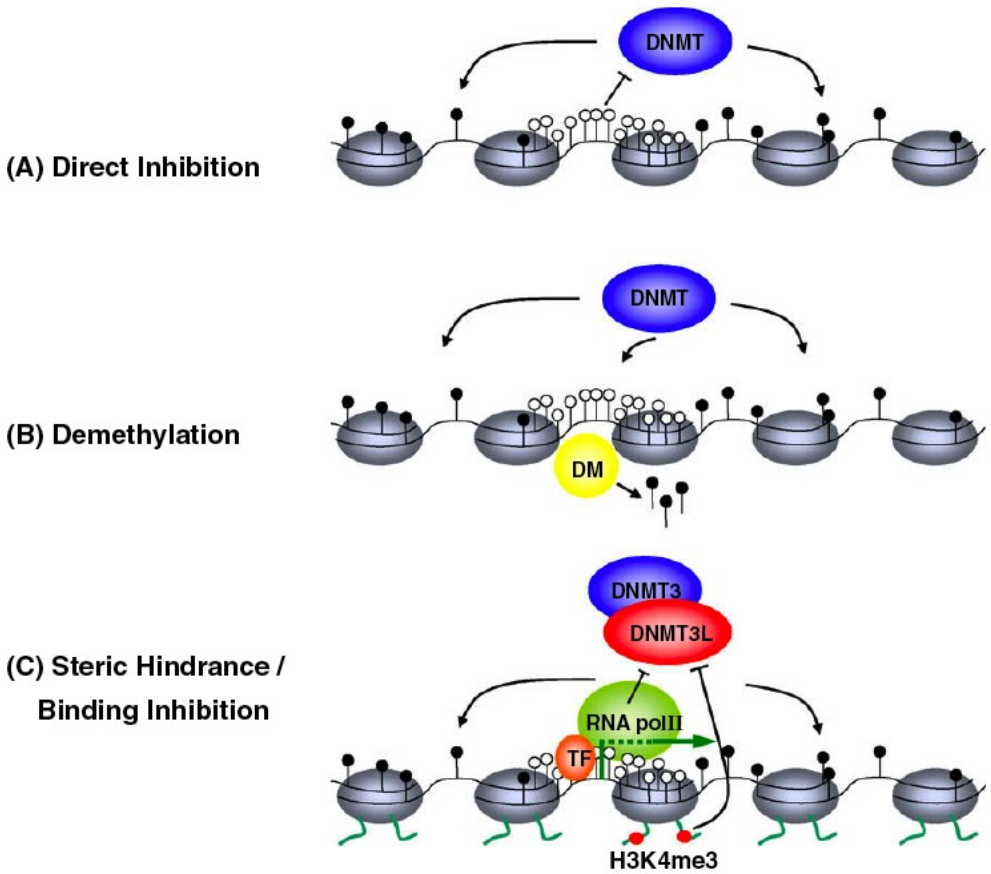
A complete pathway for dynamic modifications of Cytosine



Kohli & Zhang 2013, *Nature 502:4*

How is DNA methylation targeted?

- Mechanisms leading to CpG island hypomethylation?



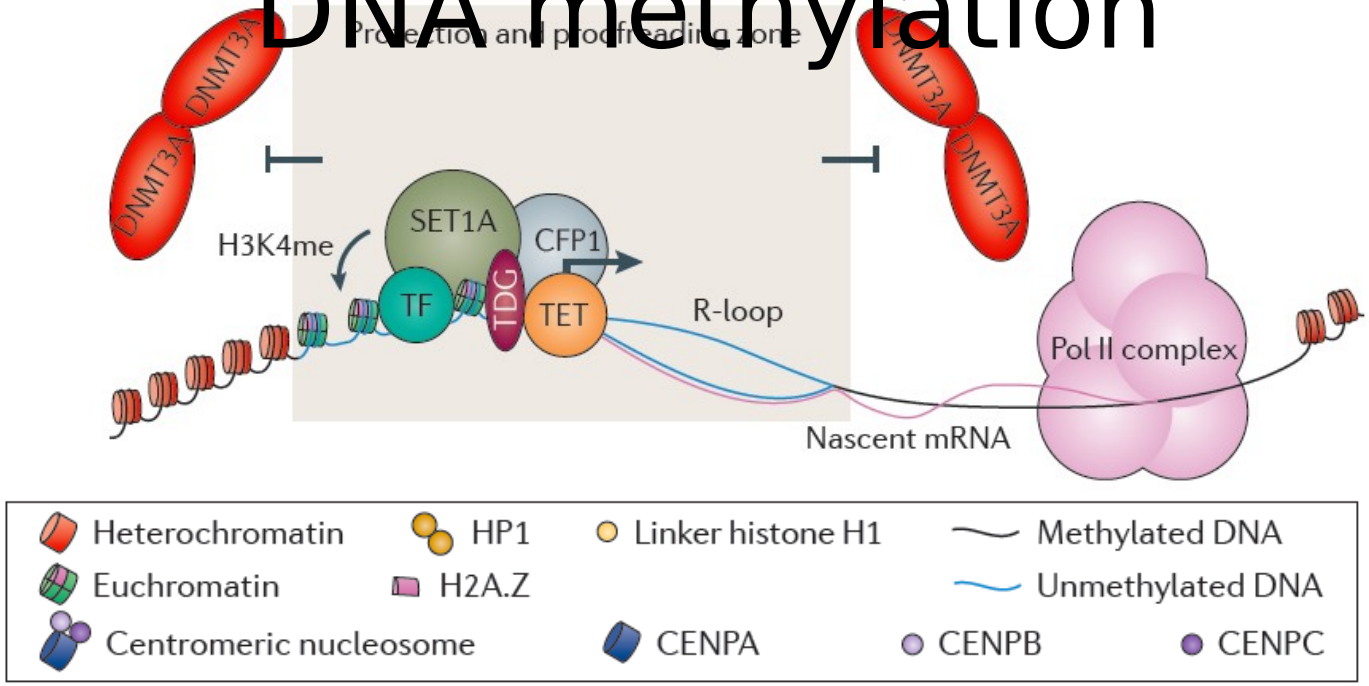
Intrinsic sequence properti

Targeting by a DNA demeth mechanism?

- **Transcription factors pre binding**
- **Dnmt3s cannot bind to H3 marked chromatin**

Illingworth & Bird (2009) *FEBS letters* **58**

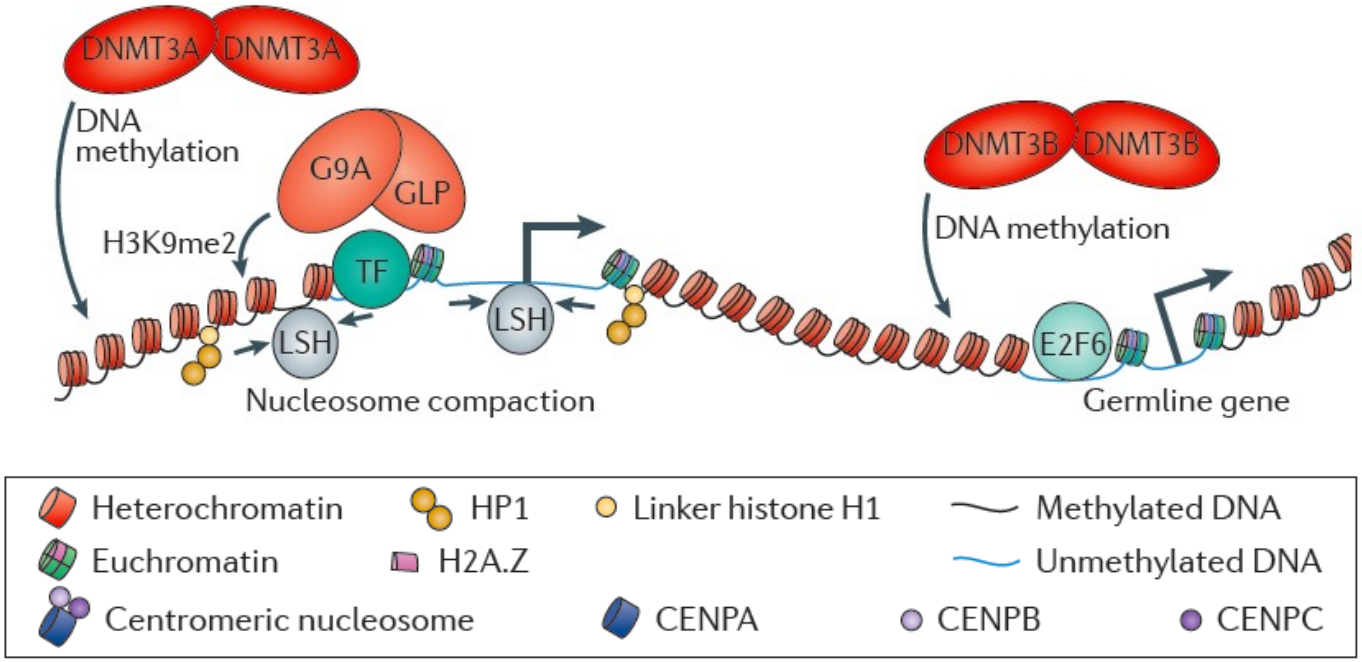
Most TSS-associated CpG islands are protected from DNA methylation



• **Components that confer protection**: TF, nucleosome exclusio
H3K4 methyltransferases (e.g. Setd1a) recruited by CFP1/MLL; DN
RNA helices inducing R-loops of ssDNA; catalytic enzymes associat
Demethylation (TET, TDG)

Smith & Meissner *2013, Nat Rev Genet 14:2*
210

Stable silencing of promoter regions

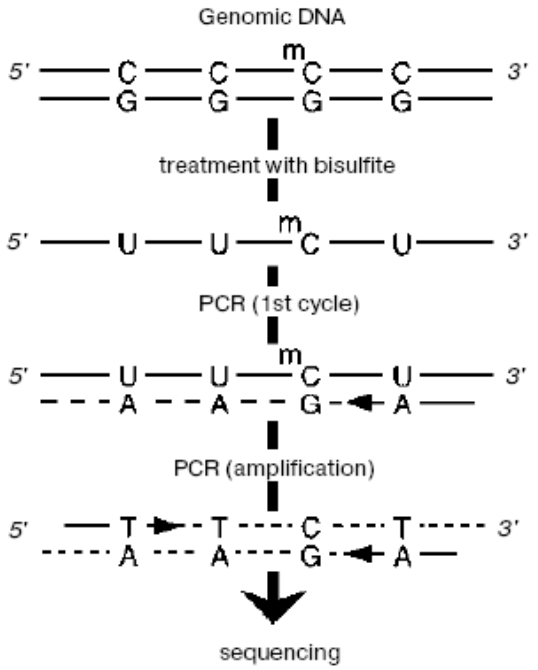


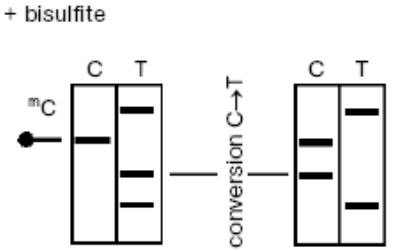
Repressive TF's directing recruitment of chromatin remodeller LSH, linker histone H1, heterochromatin protein HP1, H3K9 methyltransferases, and *de novo* methyltransferases, often in that order

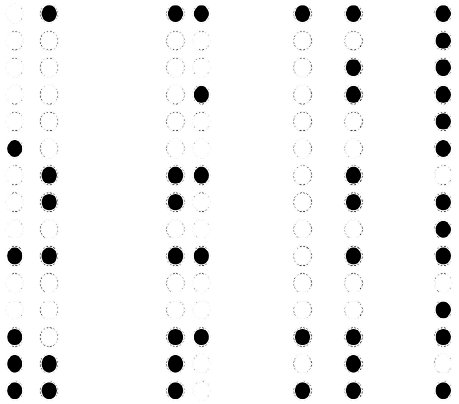
Smith & Meissner *2013, Nat Rev Genet 14:204-219*

Mapping DNA methylation

- Bisulfite







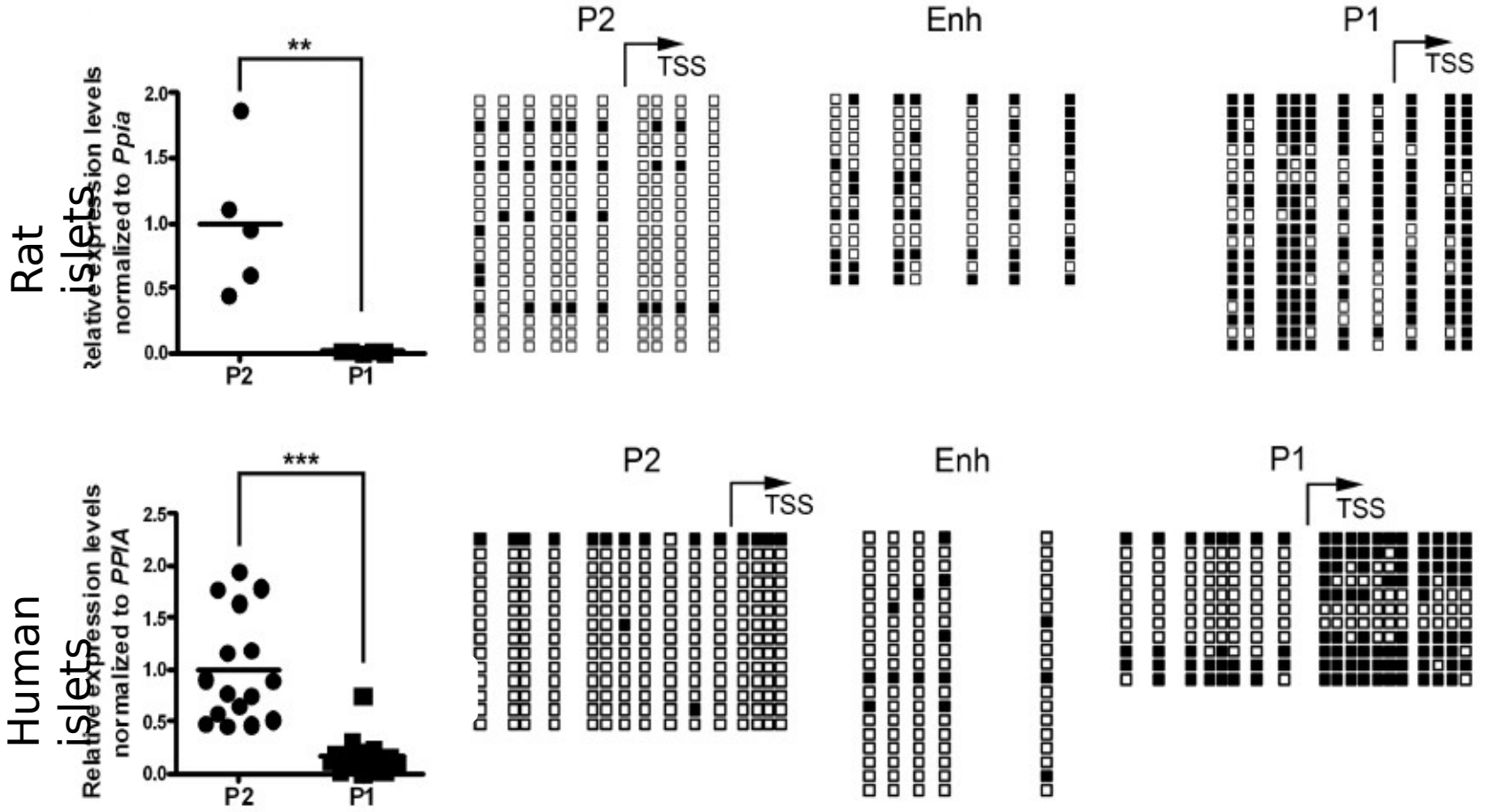
● Methylated CpG ○ Unmethylated CpG

- High-throughput Sequencing of DNA treated with bisulfite

- Other genome-wide methods include immunoprecipitation followed by arrays

mCpG cause or effect of transcriptio silencing?

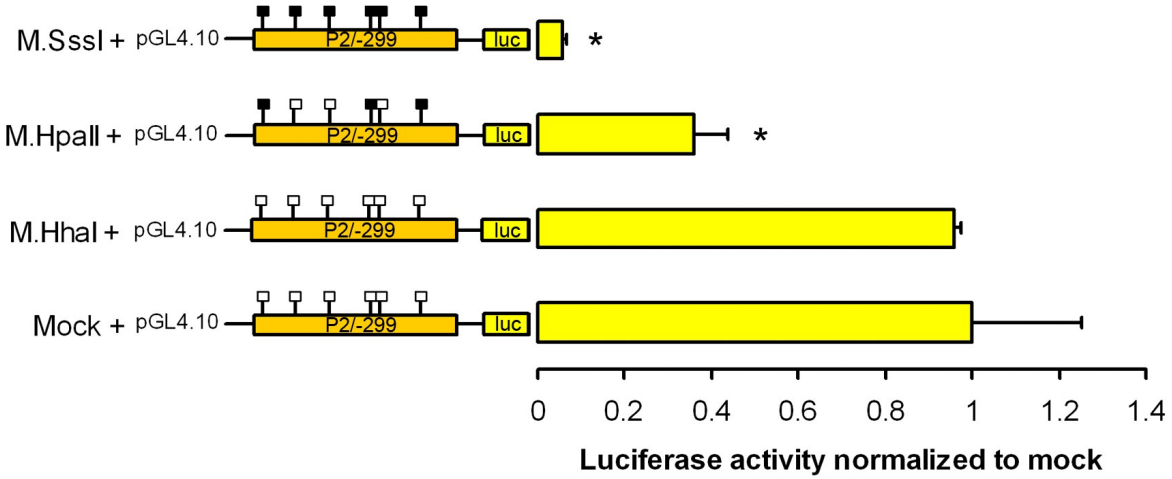
- ✓ Hnf4α is a key developmental transcription factor from the nuclear receptor superfamily



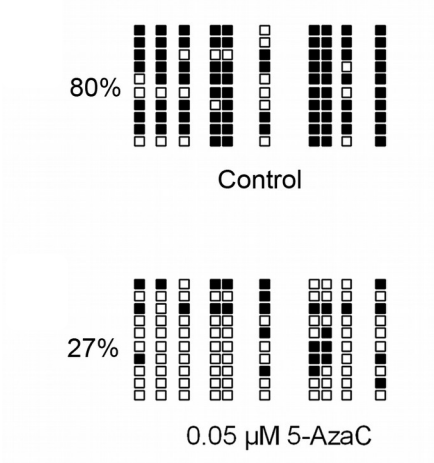
Sandovici *et al.* (2011) *Proc Natl Acad Sci* USA 108:5449-5454

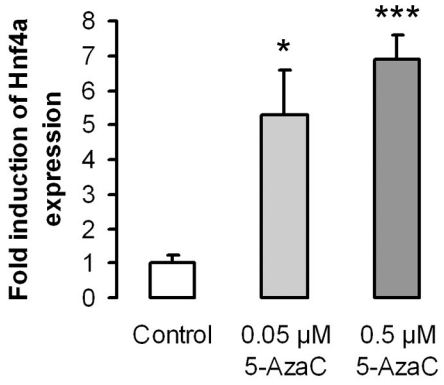
mCpG cause or effect of transcriptio silencing?

Methylation *in vitro* reduces promoter activity



Demethylation increases expression



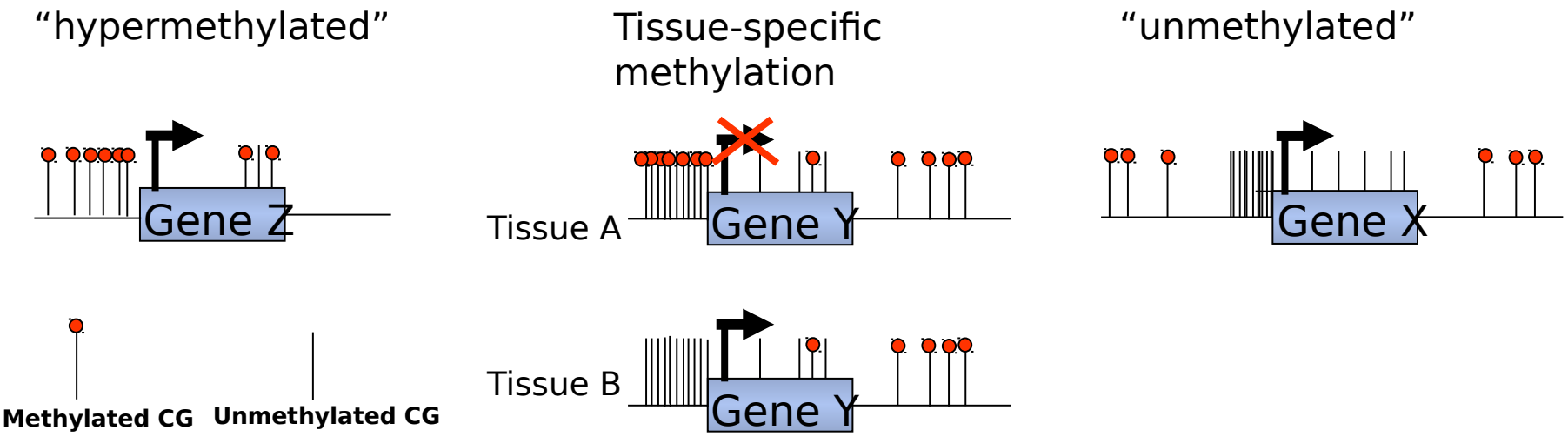


Sandovici *et al.* (2011) *Proc Natl Acad Sci* USA 108:5449-5454

Genomic patterns of DNA meth

- Most “stable” epigenetic mark
- Implicated in onset of disease
- Induced by the environment

But: Does promoter methylation “always” correlate wi gene silencing?



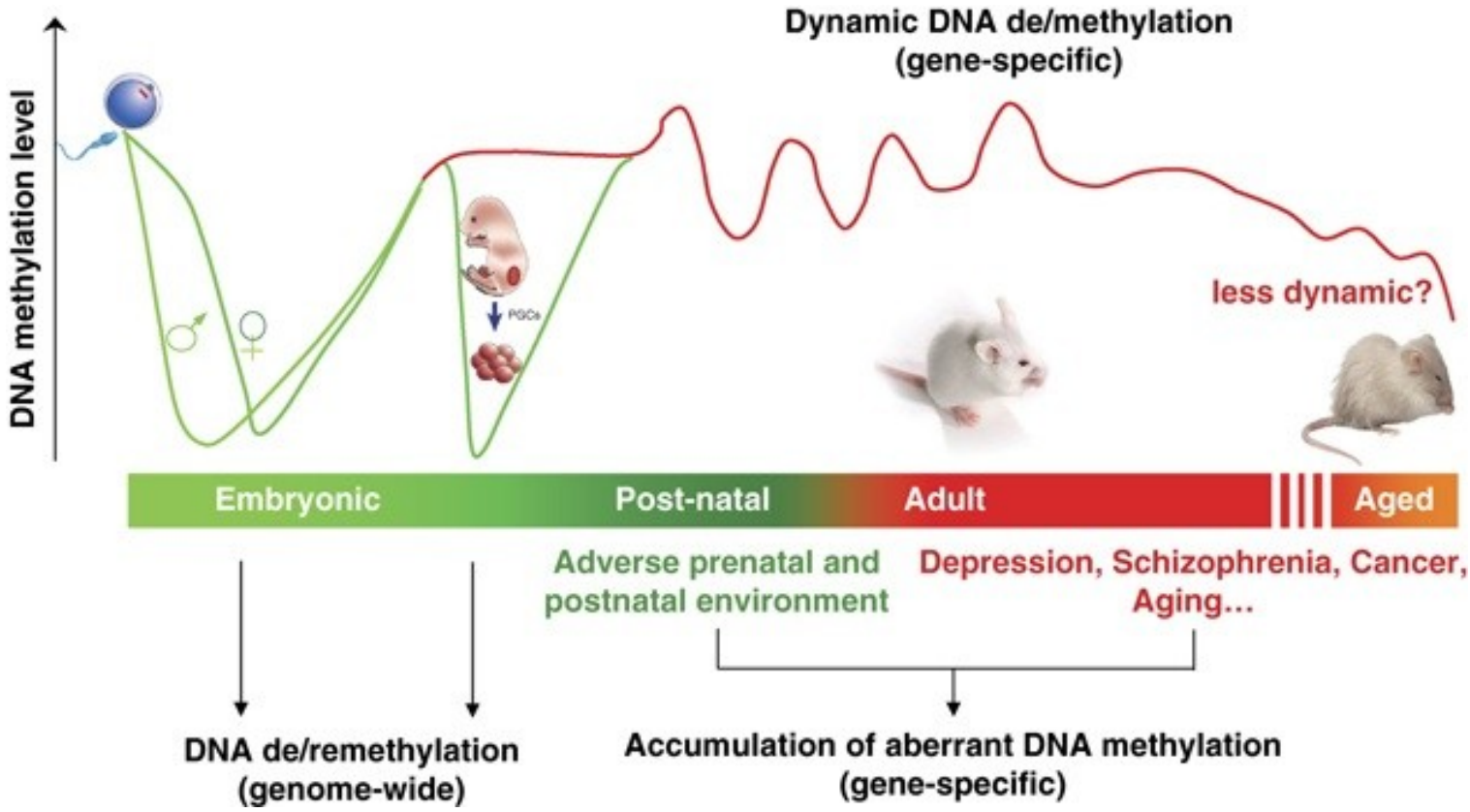
CpG poor

CpG Rich (intermediate)

CpG island

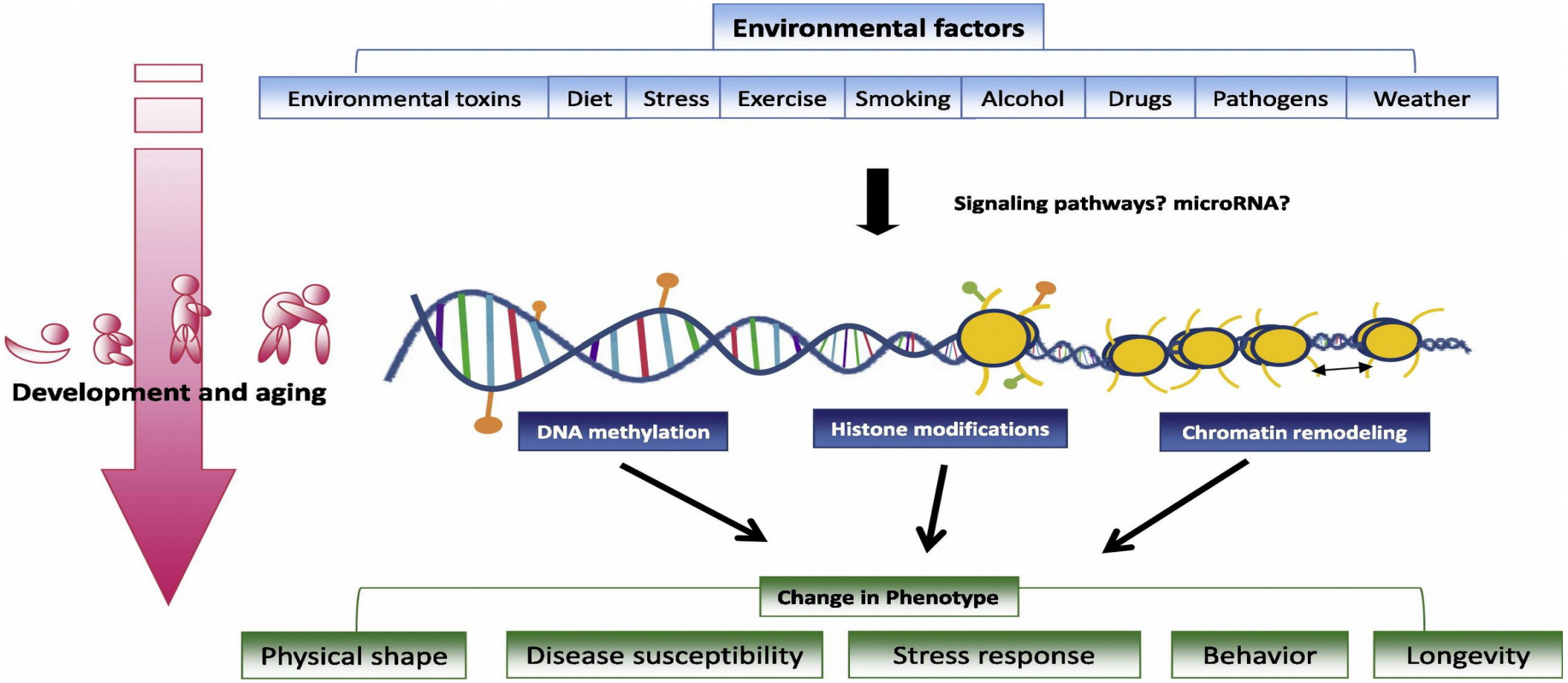
Weber *et al.* (2005) *Nat Genet* **37:**853; Weber *et al.* (2007) *Nat*

Dynamics of epigenetic information during development



Somatic epigenetic patterns need to be ‘reset’ or ‘reprogrammed in early embryos and in germ cells in order to achieve developmental pluripotency

Environmental Epigenomic



Jirtle & Skinner (2007) *Nat Rev Genet* **8:**253

Environmental Epigenomic

- Evidence that links epigenetics as potential mechanistic explanation for the long-term impact of the environment on physiology and behaviour:

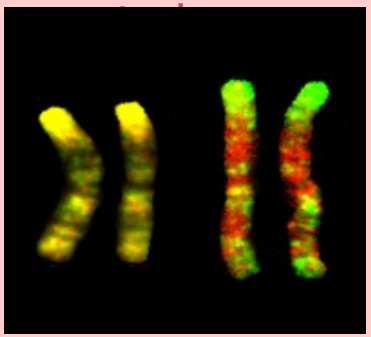
Vernalization in plants

Epigenetic responses to cold temperatures & long-term memory

Royal Jelly – the ultimate in diet evolution

Nutritional control of reproductive status in honeybees via DNA methylation

Epigenetic studies in Monozygous

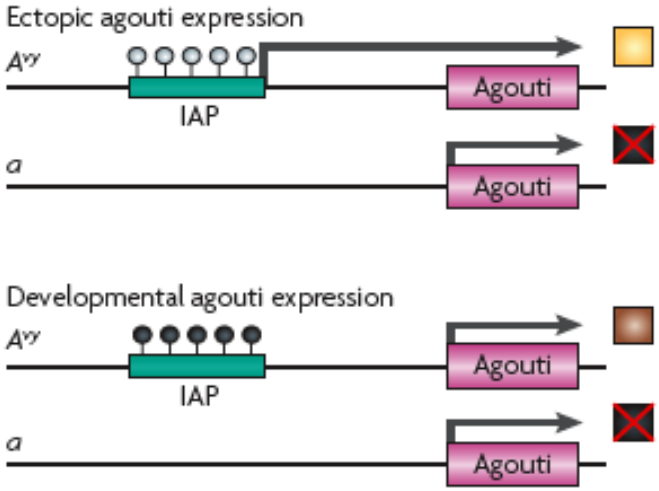


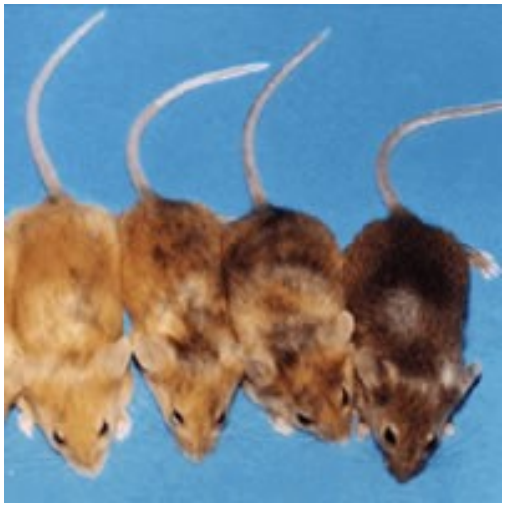
3year-old 50 year-old

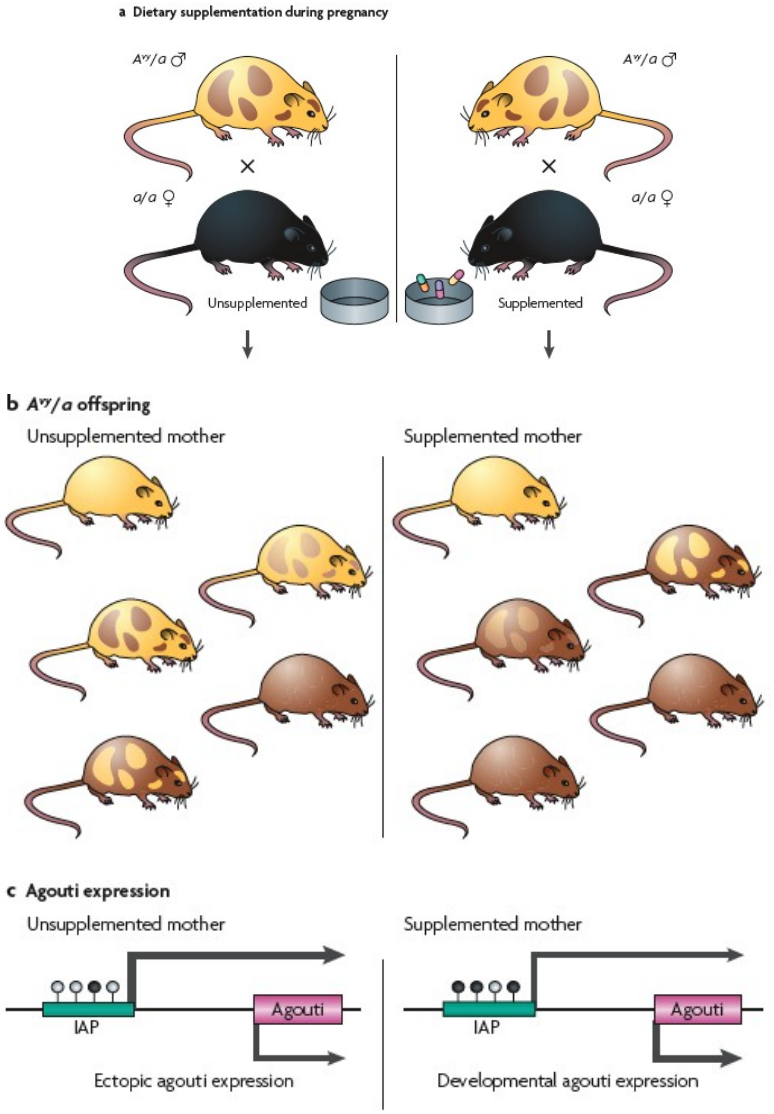
Divergent Disease

susceptibility?

Maternal nutrition in Avy mice



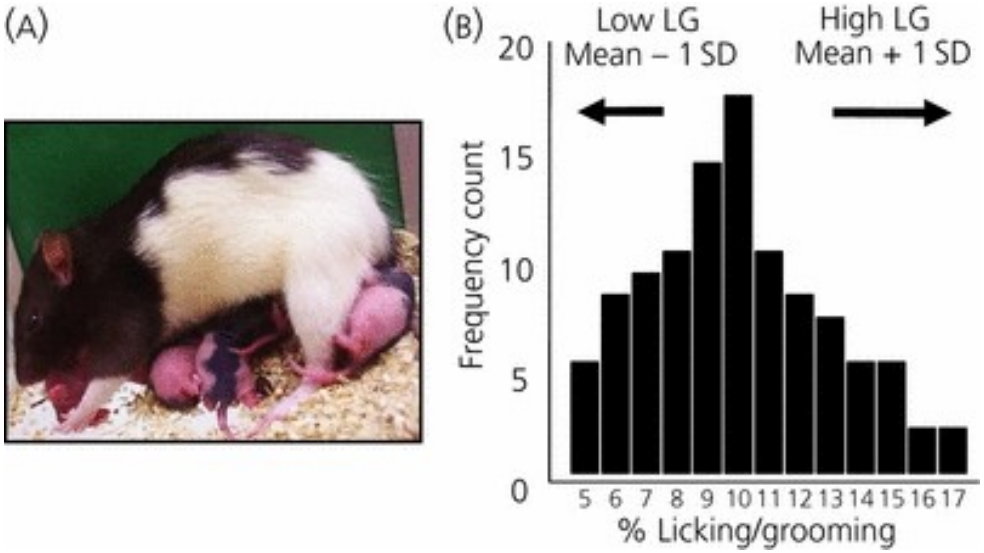




Effect of diet on DNA methylation and phenotype in offspring

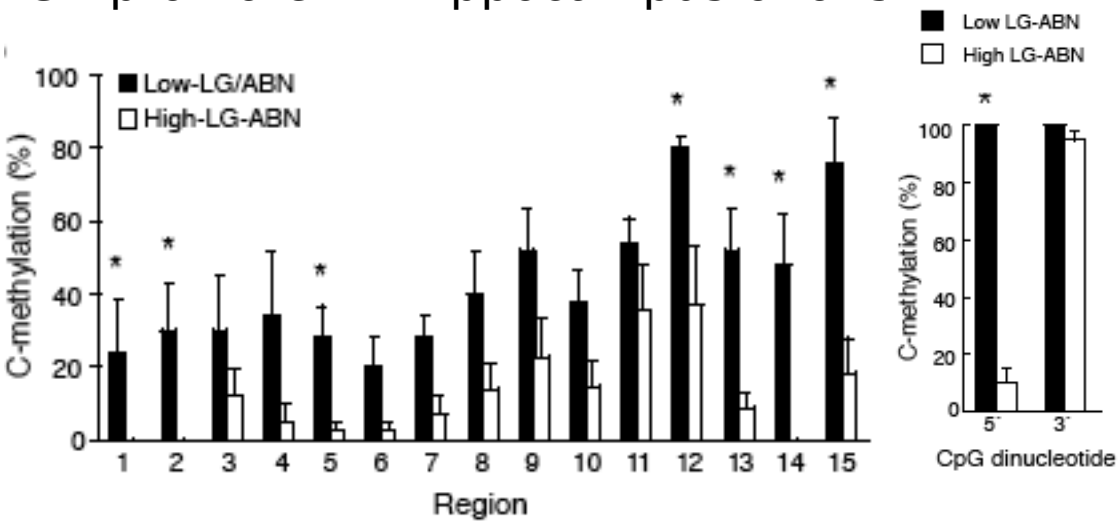
Waterland & Jirtle (2003) *Mol Cell Biol* **23**:5293

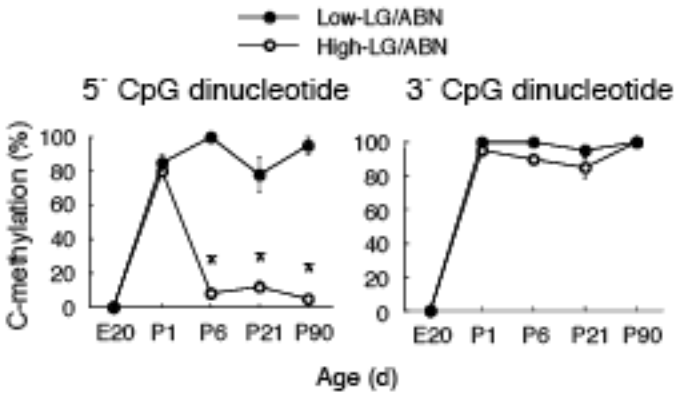
Maternal behavior



- Adult offspring of High LG ar fearful & show modest HPA response to stress
- High LG show increased hippocampal GR expression ar glucocorticoid sensitivity
- How does the effect of mater care persist into adulthood?

Maternal care alters cytosine methylation of GR promoter in hippocampus of offs





Weaver *et al.* (2004) *Nat Neurosci*

Conclusions

- CpG methylation is a mechanism of epigenetic memory
- Most CpGs in the vertebrate genome are methylated
- CpG islands are unmethylated, except for imprinted genes, X-inactivated genes, tumor-surpressor genes & key cell differentiation genes
- CpG patterns are stable in somatic cells but there is considerable dynamism during certain times in development
- Dnmt1 and Dnmt3-family proteins are responsible for establishing and maintaining global patterns of DNA methylation in mammals; active demethylation occurs by multiple mechanisms
- DNA methylation is associated with gene silencing
- Defects in DNA methylation are implicated in human disease
- Future challenges: how dynamic is DNA methylation? Is there a ‘demethylase’? Role for nonCpG methylation? 5hmC, 5fc, 5caC? Other covalent DNA modifications?